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FREEZING-POINT LOWERING OF THE LEAF SAP OF THE HORTICULTURAL TYPES OF PERSEA AMERICANA

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INTRODUCTION

In the introduction of tropical economic plants into the warmer portions of the United States, which for the most part are not free from at least occasional frosts, ability to survive transient low temperature is a characteristic of such fundamental importance that any quantitative contribution to our knowledge of its physiology must be of value. Among the factors to which frost resistance in plants is due, the magnitude of the depression of the freezing point of the cell sap has been suggested as one of importance.

That the freezing-point lowering of the cell sap is not in itself a sufficient explanation of differences in frost resistances should be quite obvious from the narrowness of its range in plants of economic importance. To what degree the osmotic pressure of the sap may be correlated with other characteristics which are of significance in cold resistance remains to be investigated.

A discussion of the several possible factors or references to the very extensive literature of the subject fall quite outside the scope of a note which is designed only to present a series of constants pertinent to the problem of hardiness in a particular tropical fruit.

The Plant Introduction Field Station at Miami, Fla., has seemed to the writers to afford particularly advantageous materials for a test of the existence of a relationship between capacity for cold resistance and the freezing-point lowering of the extracted sap, since it contains, assembled under the same environmental conditions, a wider range of varieties than can be found elsewhere.

The avocado, *Persea americana* Miller (*Persea gratissima* Gaertn. f.), while introduced to Florida and California a good many years ago, has only been propagated asexually since the beginning of the present century. Hence, the number of horticultural varieties is not overwhelmingly large. Some of these varieties have been under observation

by horticulturists for a period of 5 to 15 years, and records as to comparative hardiness have been obtained. These records show that the horticultural types differ significantly in regard to the amount of frost to which they can be subjected without injury.

The cultivated avocados fall naturally into three groups, or types, each of which possesses distinguishing characteristics which are quite constant, though occasional forms are seen which appear to be intermediate or to belong to distinct groups not yet well known in cultivation. The characteristics of the three cultivated types are briefly as follows:

MEXICAN TYPE.—Foliage and sometimes the fruit distinctly anise-scented. Leaves usually smaller than those of the Guatemalan and West Indian types. Fruit commonly 4 to 8 ounces in weight; skin thin, often membranous, usually smooth and glossy on the surface. Seed coats thin; closely united and adhering to the cotyledons, or separating, as in the West Indian type. Flowers heavily pubescent, appearing in early spring, from January to March in California and Florida. Ripe fruits from June to October. Occasionally a second crop, from later bloom, ripens in winter and spring.

GUATEMALAN TYPE.—Foliage not anise-scented, deep green, the new growth bronze-red, commonly, but not always, deeper colored than that of the West Indian type. Fruits commonly from 12 to 18 ounces in weight; skin usually verrucose or tuberculate on the surface, one-sixteenth to three-sixteenths of an inch thick, woody, brittle, and coarsely granular in texture, sharply differentiated from the flesh. Seed, as a rule, comparatively small; cotyledons smooth; the seed coats thin, closely united, and adhering to the cotyledons throughout. Flowers more finely pubescent than in the Mexican type, appearing in late spring—March to May in Florida. Fruits matured in the winter or spring of the following year.[1]

WEST INDIAN TYPE.—Foliage not anise-scented; generally similar to the Guatemalan, but the young branchlets and leaves often lighter in color. Fruits variable in form and size, as in the other types, comparatively large, averaging 14 to 20 ounces, but sometimes weighing 3 pounds or more; surface nearly always smooth, the skin rarely more than one-sixteenth of an inch thick, pliable and leathery, and scarcely so well differentiated from the flesh as in the Guatemalan. Seed usually large in proportion to the size of the fruit; cotyledons more or less rough; the two seed coats frequently thick and separated, at least over the distal end of the seed, one adhering to the cotyledons and the other loose or adhering to the wall of the seed cavity. Flowers usually as pubescent as those of the Guatemalan type, occasionally glabrate; in Florida and the West Indies they appear from February to April. Matured fruits from July to November.

[1] Some of the varieties of this type have the skin nearly smooth and about the same thickness as that of the West Indian. For this reason they have been considered by a few horticulturists as forming a distinct class; but inasmuch as they seem to differ only in these two points, which are both variable characters at best, it seems safe to retain them in the Guatemalan type.

EXPERIMENTAL METHODS

The technique employed in determining freezing-point lowering was very simple. Fully matured but still normally green leaves were collected and frozen in an ice and salt mixture to facilitate the extraction of sap by pressing[1] in a small heavily tinned bowl under a powerful screw. The freezing-point lowering was determined by means of a mercury thermometer graduated to 0.01 of a degree centigrade in subdivisions sufficiently large to allow of fairly accurate estimation to smaller fractions. The vaporization of ether or carbon bisulphid in a vacuum jacket was used in determining the freezing-point lowering. The results are expressed in terms of freezing-point lowering in degrees centigrade (Δ), corrected for undercooling by the usual formula.

For those who prefer to think in terms of osmotic pressure the values of P are given from a published table.[2] Finally, for the convenience of those who wish to know the actual freezing point of the saps on the Fahrenheit scale, these values have been added (F).

PRESENTATION OF CONSTANTS FOR THE THREE TYPES[3]

MEXICAN TYPE.—Throughout the highlands of central and northern Mexico this type is very common. Because of its superior hardiness, it has been extensively planted in several subtropical regions, most notably in California and Chile. It is known to have been planted in California as early as 1870. In Florida it has fruited as far north as Gainesville, but is not generally cultivated in any part of the State. It has been planted along the Riviera in southern Europe and has fruited in the open at Rome. In Algeria it has also been planted, though to a very limited extent. In the West Indies it is scarcely known.

During the cold weather of January, 1913, in California trees of this type were reported to have withstood temperatures of 16° to 20° F. without injury.

1. Querétaro..... $\Delta=1.11$, $P=13.3$, $F=30.00°$
Buds received from California, where the variety was introduced from Querétaro, Mexico, in 1911. Cion 1 year old, on one branch of No. 4, Mexican stock.
2. San Sebastian..... $\Delta=1.20$, $P=14.5$, $F=29.84°$
Buds received from California, where the variety was introduced from Querétaro, Mexico in 1911. One-year-old cion on Mexican stock, a branch of No. 4.
3. Harman..... $\Delta=1.24$, $P=15.0$, $F=29.77°$
Originated at Sherman, Cal., budded on a limb of 26713, West Indian type. Cion 2 years old.
4. Seedling..... $\Delta=1.27$, $P=15.2$, $F=29.71°$
Grown from seed of unknown origin.

[1] Gortner, R. A., and Harris, J. A. Notes on the technique of the determination of the depression of the freezing point of vegetable saps. *In* Plant World, v. 17, No. 2, p. 49–53, 1914.

[2] Harris, J. A., and Gortner, R. A. Notes on the calculation of the osmotic pressure of expressed vegetable saps from the depression of the freezing point, with a table for the values of P for $\Delta=0.001°$ to $\Delta=2.999°$. *In* Amer. Jour. Bot., v. 1, no. 2, p. 75–78. 1914.

[3] The numbers following the varietal names refer to the Inventory of the Office of Foreign Seed and Plant Introduction, Bureau of Plant Industry.

5. Harman.....Δ=1.29, *P*=15.6, *F*=29.68°
Originated at Sherman, Cal. Two-year-old cion on one branch of No. 4, Mexican stock.

6. Seedling (19206).....Δ=1.30, *P*=15.7, *F*=29.66°
Grown from seed received from Coahuila, Mexico.

7. Seedling.....Δ=1.30, *P*=15.6, *F*=29.66°
Grown from seed of unknown origin.

8. Seedling.....Δ=1.30, *P*=15.6, *F*=29.66°
Grown from seed of unknown origin.

9. Seedling.....Δ=1.36, *P*=16.3, *F*=29.55°
Grown from seed of unknown origin.

10. Seedling (34831).....Δ=1.37, *P*=16.5, *F*=29.53°
Budded tree from a seedling growing in Rome, Italy.

11. Seedling (32400).....Δ=1.39, *P*=16.7, *F*=29.50°
Originated at Orange, Cal. Budded tree.

12. Fuerte.....Δ=1.41, *P*=16.9, *F*=29.46°
Buds received from California, where the variety was introduced in 1911 from State of Puebla, Mexico. One-year-old cion on branch of No. 33, West Indian stock.

13. Seedling.....Δ=1.43, *P*=17.2, *F*=29.43°
Grown from seed of unknown origin.

GUATEMALAN TYPE.—This group was first called to the attention of horticulturists by Collins in 1905.[1] Within the last few years it has been extensively planted in California and is now becoming known in Florida. It is found commonly in the mountainous parts of Guatemala and northward into southern Mexico, whence have come many of the cultivated varieties now being propagated in the United States. It was introduced to Hawaii about 20 years ago, according to Higgins, Hunn, and Holt,[2] while it appears to have been first planted in California about 1885. In Florida it was probably not introduced earlier than 1900. It has not been observed in the West Indies, with the exception of a few trees recently planted, and its distribution in other countries is quite limited.

In California, where it is best known horticulturally, it has been found considerably hardier than the West Indian type, but somewhat more tender, as a rule, than the Mexican.

14. Seedling (10978).....Δ=1.09, *P*=13.1, *F*=30.04°
Grown from seed introduced from Guatemala in 1904.

15. Colorado.....Δ=1.16, *P*=13.9, *F*=29.91°
Budded on one limb of No. 24, West Indian type. Originated at Los Angeles, Cal., the seed having been sent from the State of Puebla, Mexico. Cion 1 year old.

16. Taft.....Δ=1.25, *P*=15.0, *F*=29.75°
Originated at Orange, Cal. Two-year-old cion on limb of No. 4, Mexican stock.

17. Seedling (38549).....Δ=1.34, *P*=16.2, *F*=29.59°
Originated at Antigua, Guatemala. Budded on one limb of (26694), West Indian type.

18. Sinaloa.....Δ=1.35, *P*=16.2, *F*=29.57°
Buds obtained from California, where the variety was introduced in 1911 from the State of Puebla, Mexico. Cion 1 year old, on limb of No. 27, West Indian type.

[1] Collins, G. N. The avocado, a salad fruit from the Tropics. U. S. Dept. Agr. Bur. Plant Indus. Bul. 77, 52 p., 8 pl. 1905.

[2] Higgins, J. E., Hunn, C. J., and Holt, V. S. The avocado in Hawaii. Hawaii Agr. Exp. Sta. Bul. 25, 48 p., illus., 7 pl. 1911.

19. Nutmeg.....Δ=1.38, *P*=16.5, *F*=29.52°
Buds obtained from Hawaii, where the variety originated from a seed received from Guatemala. Two-year-old cion on West Indian stock.
20. Seedling (36603).....Δ=1.38, *P*=16.6, *F*=29.52°
Budded tree. Original grown at Honolulu, Hawaii, from a seed imported from Guatemala.
21. Seedling (19080).....Δ=1.39, *P*=16.8, *F*=29.50°
Tree introduced as a plant from Guatemala in 1906.
22. Seedling (19058).....Δ=1.40, *P*=16.8, *F*=29.48°
Grown from seed introduced from Guatemala in 1906.

WEST INDIAN TYPE.—This is the commonest type on tropical American seacoasts. Its precise origin is not known, but since it has been demonstrated by Collins[1] that the avocado was not cultivated in the West Indies in pre-Columbian times it must have been introduced to the islands from some point on the mainland. At the present day it is practically the only type grown in the West Indies, and it is known to be common in Colombia, Venezuela, Brazil, and Peru. It occurs in the Mexican lowlands and in Yucatan. Most of the avocados grown in Hawaii belong to this type, as do those of Tahiti, some of which are occasionally shipped to San Francisco. In south Florida it is the principal type cultivated, having been introduced probably from Cuba at an early day and planted on both the east and west coasts. In California its cultivation is very limited. It is found in the Canary Islands and has been introduced to India, but in this latter country avocado culture is of no importance.

Horticulturally the most important feature of this type is its susceptibility to cold, which prevents its culture in any but the warmest sections of the United States. Experience has shown it to be severely injured by a temperature at least 4 or 5 degrees Fahrenheit higher than that required to injure the Guatemalan type.

23. Seedling (26707).....Δ=0.96, *P*=11.6, *F*=30.27°
Originated at Fulford, Fla. Budded tree.
24. Seedling (26692).....Δ=1.00, *P*=12.0, *F*=30.20°
Originated at Santiago de las Vegas, Cuba. Budded tree.
25. Seedling (26693).....Δ=1.03, *P*=12.4, *F*=30.15°
Originated at Cocoanut Grove, Fla. Budded tree.
26. Seedling (26704).....Δ=1.03, *P*=12.4, *F*=30.15°
Originated at Miami, Fla. Budded tree.
27. Largo (18730).....Δ=1.04, *P*=12.5, *F*=30.13°
Originated at Nassau, Bahama Islands. Budded tree.
28. Seedling (26698).....Δ=1.04, *P*=12.5, *F*=30.13°
Originated at Fort Myers, Fla. Budded tree.
29. Seedling (19297).....Δ=1.06, *P*=12.8, *F*=30.09°
Originated at Cocoanut Grove, Fla. Budded tree.
30. Seedling (36270).....Δ=1.07, *P*=12.9, *F*=30.07°
Grown from seed of unknown origin.
31. Seedling (26703).....Δ=1.08, *P*=12.9, *F*=30.06°
Originated at Buena Vista, Fla. Budded tree.

[1] Collins, G. N. Op. cit.

32. Seedling (26694).....Δ=1.09, *P*=13.2, *F*=30.04°
Originated at Marco, Fla. Budded tree.
33. Seedling.....Δ=1.09, *P*=13.1, *F*=30.04°
Grown from seed of unknown origin.
34. Butler (26690).....Δ=1.10, *P*=13.3, *F*=30.02°
Originated at St. Petersburg, Fla. Budded tree.
35. Seedling (26691).....Δ=1.15, *P*=13.9, *F*=29.93°
Originated at Buena Vista, Fla. Budded tree.
36. Mitchell (18120).....Δ=1.19, *P*=14.3, *F*=29.86°
Originated at Bayamon, Porto Rico. Budded tree.
37. Seedling (26713).....Δ=1.23, *P*=14.8, *F*=29.79°
Originated at Cocoanut Grove, Fla. Budded tree.
38. Seedling (19379).....Δ=1.23, *P*=14.8, *F*=29.79°
Grown from a seed received from Hawaii.
39. Pollock.....Δ=1.24, *P*=14.9, *F*=29.77°
Originated at Miami, Fla. Budded tree.

DISCUSSION OF CONSTANTS

All these types show considerable variation in freezing-point lowering. For the whole series the range is from Δ=0.96° to Δ=1.43°, or 0.47°. On the Fahrenheit scale the range in the freezing point of the sap is from 29.43° to 30.27°. This variation is doubtless due to many causes. In addition to errors of sampling in the collection of the leaves from the individual trees and the unavoidable errors of measurement involved in the manipulation of the sap samples, there are the fluctuations attributable to the uncontrollable differences in the physiological states of the different trees at the time of collecting the materials.

In view of these various factors, one would not expect to find transition types entirely wanting, unless the differentiation of the types be very clearly marked indeed. For more convenient comparison the values obtained from the trees of the three types may be serially arranged in intervals of five-hundredths of a degree (Table I).

TABLE I.—*Comparison of the freezing-point lowering values of three types of Persea americana*

Depression of freezing point.	Guatemalan type.	Mexican type.	Guatemalan and Mexican types.	West Indian type.
° *C.*				
0.91–0.95	.....	.....	.....	.....
0.96–1.00	.....	.....	.....	2
1.01–1.05	.....	.....	.....	4
1.06–1.10	1	.....	1	6
1.11–1.15	.....	1	1	1
1.16–1.20	1	1	2	1
1.21–1.25	1	1	2	3
1.26–1.30	.....	5	5	.....
1.31–1.35	2	.....	2	.....
1.36–1.40	4	3	7	.....
1.41–1.45	.....	2	2	.....
1.46–1.50	.....	.....	.....	.....
	9	13	22	17

An inspection of the values in Table I shows no clear difference between the plants of the Guatemalan and Mexican types. From both of these the West Indian type seems to be differentiated by a distinctly slighter freezing-point lowering. All but 3 of the 17 West Indian determinations are lower than 1.20°, whereas all but 4 of the 22 based on plants of the Guatemalan and Mexican types show a freezing-point lowering of 1.21° or more.

If the averages of the freezing-point lowerings (Δ) with their probable errors be determined as a more exact means of comparison from the ungrouped depressions given for the individual varieties above, the following constants are obtained (Table II).

TABLE II.—*Freezing-point lowering constants of three types of Persea americana*

Type.	Mean.	Standard deviation.	Coefficient of variation.
Guatemalan	1.304±0.024	0.106±0.017	8.16
Mexican	1.305± .016	.087± .012	6.68
Guatemalan and Mexican	1.305± .014	.095± .014	7.25
West Indian	1.096± .014	.083± .010	7.55

The average freezing-point lowering in the Guatemalan and Mexican types is practically the same. The difference is only 0.001 ±0.029 of a degree. The West Indian type is characterized by a distinctly lower average than either of the other types. The differences are:

West Indian and Guatemalan type	−0.209±0.027°
West Indian and Mexican type	−0.210±0.021°
West Indian and Guatemalan and Mexican types	−0.209±0.019°

These differences are 7.6, 9.9, and 11 times as large as their probable errors. Thus, notwithstanding the relatively small number of observations upon which this study has of necessity been based, the differences seem to be quite trustworthy in comparison with their probable errors.

Within the type the absolute variation in freezing-point lowering is very slight, amounting to one-tenth of 1 degree or less. The relative variability as expressed in terms of the coefficient of variation is also low for a plant character.

SUMMARY

The constants presented in this paper prove that in a tropical fruit of relatively recent introduction to North American horticulture, the avocado, one of the groups of varieties, the so-called West Indian type, is characterized by tissue fluids which freeze at a distinctly higher temperature than in the two other groups of varieties (Guatemalan and Mexican). In the conventional terms of physical chemistry adopted by physiologists, the expressed leaf sap of West Indian type varieties is characterized by a slighter depression of the freezing point or by a slighter freezing-point lowering than is that of the two other groups of

varieties. This differentiation seems to hold with remarkable constancy notwithstanding the wide geographic origin—West Indian, Bahaman, Central American, Mexican, and Hawaiian—of the seeds or budwood from which the tissues dealt with originated.

The type which is characterized by the slightest freezing-point lowering of its extracted sap—that is, the type in which the expressed sap freezes at the highest temperature—is the one which has been shown by horticultural experience to be the least capable of enduring cold. That capacity to withstand low temperatures is not solely due to differences in the freezing point of the sap is evident from the slightness of the differences in the cryoscopic constants of the West Indian as compared with the Mexican and Guatemalan types. Furthermore, horticulturists believe that the plants of the Guatemalan type are intermediate in hardiness between those of the Mexican and West Indian types. There is, so far as our data go, no discernible difference in the freezing point of the sap of these types.

The problem is evidently one of considerable complexity. To what extent other characteristics contributing to the capacity of the organism to withstand low temperatures are correlated with sap properties remains to be investigated.

It seems highly probable from the evidences presented in the paper that in the case of the tropical perennials, a knowledge of the freezing-point lowering of the sap would be of some service in predicting ability to withstand cold. At least the subject is one deserving of more extensive investigation. We would have been glad to carry out the present study on a far more extensive scale, but the determinations given practically exhaust the trees of flowering age in the collection of Guatemalan and Mexican types at the Miami Plant Introduction Field Station, and it will probably be several years before a better series is available.

GRAIN OF THE TOBACCO LEAF

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INTRODUCTION

When the buyer examines a sample of cigar leaf, he takes into account such factors as texture, elasticity, color, luster, thickness, and grain. He rolls a trial cigar and considers the burn, flavor, aroma, and character of the ash. These he judges through long experience and by comparison with an arbitrary standard for the crop of that particular year and the type of leaf in determining the price he will pay for the tobacco of which the sample is representative. Though the character of the grain itself, whether it be fine or coarse, close or open, may be a relatively minor consideration in fixing the value of a given sample, it is undoubtedly closely correlated with some of the other factors considered and is therefore of more importance than has hitherto been supposed.

OCCURRENCE AND GENERAL MACROSCOPIC APPEARANCE OF THE GRAIN

The usual form of the grain of tobacco appears to the unaided eye as minute pimples or papillæ slightly raised above the general surface of the leaf tissue. They are more prominent on the upper surface and vary in size from about 1 mm. in diameter down to bodies so small as to be entirely invisible without the aid of the microscope. When a well-cased leaf is stretched over the ball of the finger, the grain becomes more conspicuous (Pl. 15, fig. A and B), and, indeed, it is in this way that the tobacco buyer or grader determines the size and distribution of these bodies. If a leaf with a coarse grain be pressed between the thumb and finger, it feels as though particles of sand were adhering to it. Some of the larger grain bodies can be picked out with the point of a knife; and when such a grain is pressed between two hard substances, it crushes in the same manner as would a particle of crystal, for the grain itself is a crystalline body. On burning, these grain bodies swell and cause the pearl-like pimples so frequently seen on the ashes of cigars (Pl. 15, fig. C and D). Grain is a characteristic of cigar tobacco, both domestic and imported (7).[2] In the flue-cured types macroscopically visible crystalline deposits seem to be entirely absent. However, it is reasonable to suppose that all air-cured types possess grain in some degree.

[1] The writer wishes to express his gratitude for helpful suggestions and kind criticisms made by Dr. W. W. Garner, for inorganic and organic analyses made by Mr. C. L. Foubert and Dr. C. W. Bacon, respectively, and for kind cooperation on the part of Mr. Otto Olson in the matter of obtaining material.

[2] Reference is made by number to "Literature cited," p. 273.

With two notable exceptions the grain particles are entirely embedded in the tissue of the leaf, being distributed more or less evenly in the web and along the veins. One of the superficial types appears as raised black dots, which are found chiefly on dark-colored leaves of a heavy texture. The other is composed of those which give somewhat the appearance of minute disks situated on or immediately beneath the surface on either side of the leaf. These latter seem most conspicuous on leaves of a medium or light color.

MICROSCOPIC CHARACTERS OF THE GRAIN

When a cased leaf of tobacco containing grain is stretched over the stage of the microscope and examined in ordinary transmitted light with a low magnification, most of the larger grain particles appear as more or less rounded, highly refractive bodies of compact structure and bright reddish brown color. Indeed, the ground tissue appears uniformly light in color in comparison with that of the leaf observed macroscopically, and it is seen that by far the larger part of the brown color of the leaf is localized in the grain and in some of the small veins. Even when one looks with a hand lens through a moist leaf against a strong source of light, this localization of color in the grain is evident; the effect is that of minute garnets embedded in a yellowish brown matrix.

In material mounted in Canada balsam, after having been dehydrated in alcohol and cleared in xylol, the grain bodies become less conspicuous in ordinary transmitted light, inasmuch as their refractive index more nearly approaches that of the balsam than that of the air. The bodies may be located, however, because of their difference in color from the surrounding ground tissue, owing to the concentration in them of the brown coloring matter and their slight power of refraction of light in balsam (Pl. 16, fig. A).

It is in polarized light that the grain bodies become most conspicuous, since, as stated before, they are of crystalline structure. Favorable material mounted in balsam then exhibits clearly their size, shape, and structure, as well as showing the degree to which the grain material has been brought together into definite aggregates, a point which will be mentioned later in this paper.

VARIOUS FORMS OF GRAIN

There are five general types of grain which merge insensibly into one another and which may be briefly described in their order of abundance, as follows: The first and most common type consists of more or less spherical masses each composed chiefly of a group of palisade cells distended with minute, radiating, needle-shaped crystals of a brown color (Pl. 16, fig. B). The second type includes flat or roughly hemispherical bodies composed of cells of the mesophyll and epidermis, which are filled with light brown to almost colorless, comparatively large plates arranged somewhat regularly (Pl. 16, fig. C). The third is similar to the

first type, in that the bodies are usually spherical; but here the surface is decidedly nodular and the cells included may be either the palisade or the spongy parenchyma, or both. The crystals are radially arranged in small groups the individuals of which appear to be thin, narrow plates, and the color is more gray than brown (Pl. 16, fig. D).

The fourth type attains the largest size and always has one surface in common with the surface of the leaf. The particles consist of a number of cells, palisade and epidermal, filled with a mass of dark-brown or black substance which in the unbroken particle is inactive in polarized light. When crushed, however, the fragments between crossed Nicol prisms show the presence of crystalline material the form of which is not apparent. These dark bodies are very striking, in that the epidermal surface is usually craterlike in appearance, having a concavity which frequently contains a few minute, colorless crystals surrounded by a raised black ring. In a few of this type the central portion of the upper surface shows no concavity, but radiating lines extending from a centrally located spot beneath which the substance of the particle is soft and easily crumbled suggest that this spot marks the location of the base of one of the large trichomes or, possibly, the position of a stoma which failed to close during the curing process (Pl. 16, fig. E).

The fifth type of grain is composed entirely of microscopic sphere crystals which are very active in polarized light and plainly show the four extinction bars which rotate upon revolving the analyzer. Unlike the other types, they usually do not fill the cells in which they occur. They are colorless or light brown in ordinary transmitted light and possess no visible differentiation into individual crystals (Pl. 16, fig. F). Tunmann (8, p. 147) indicates that these spherites, which he believes to be malic-acid salts, separate out when dried tobacco is placed in alcohol. The writer has observed that they are formed in the leaf during the process of curing and that in some instances they are visible under the microscope in the untreated, dry leaf with the aid of strong polarized light, even after fermentation has been completed.

OTHER CRYSTALLINE MATERIAL OF THE LEAF

Aside from the grain, two other types of crystalline material are found in the tissue of the cured and fermented tobacco leaf. One of these is the cryptocrystalline or sand crystals of calcium oxalate contained in certain cells (idioblasts) which are always present in all tobacco, even in the green leaf while it is still attached to the growing plant, and which show no appreciable change during the process of curing or fermentation. The other is that which appears in nearly every cell of the leaf in both green and cured tobacco and consists of small, single prisms. Some of the properties of both of these types will be mentioned in connection with a consideration of some of the chemical characteristics of the grain (Pl. 16, fig. A, and Pl. 17, fig. A).

METHOD OF SEPARATION OF GRAIN FROM THE LEAF AND MECHANICAL ANALYSIS OF THE LEAF

Preliminary investigations indicated that a considerable supply of the grain bodies free from the surrounding tissues of the leaf would be desirable. A method of mechanical separation was therefore worked out, which, in brief, is as follows: A quantity of air-dry cigar leaf tobacco was rubbed through a series of sieves (from 10 to 150 mesh) with a flat pestle made from a rubber stopper. This process removed a large amount of the soft web of the leaf from the veins as well as from the hard grain particles, at the same time breaking the veins into short lengths, but not crushing the grain. The mixture of grain and vein remaining in each sieve was then ground in an unglazed porcelain mortar with a rounded rubber pestle. This served to remove any soft tissue still adhering to the particles of grain and vein. After sifting out with the appropriate sieve the small amount of fine material thus rubbed off, the mixture of grain and vein remaining was slowly poured upon a piece of smooth paper inclined at an appropriate angle, determined by experiment. The more or less spherical particles of grain rolled down the inclined paper more rapidly than the cylindrically-shaped pieces of vein, resulting in a partial separation of the former from the latter. It was found, however, that a large amount of the grain belonging to the flatter types remained with the vein, and it was therefore necessary to pick this grain out of the mixture with the aid of a binocular microscope. By these tedious means about 30 or 40 gm. of practically pure grain were obtained for subsequent detailed study.

The separation of the grain and vein from the soft tissues of the leaf in this manner in one instance also resulted in an approximate mechanical analysis of the tobacco leaf. It was found that of a 70-gm. sample the midribs represented about 33 per cent of the weight, while the soft tissue (that which passed through a 150-mesh sieve) constituted 48 per cent; the veins (other than the ribs), 8 per cent; and the grain, 11 per cent. Excluding the ribs, the soft tissue represented 70 per cent; the veins, 12 per cent; and the grain, 17 per cent by weight. These results are only approximate, since the primary object of the separation was to obtain a quantity of the pure grain, and the determination of the proportion of various tissues was an afterthought. It seems probable, however, that an improved method for the accurate mechanical analyses of tobacco would be highly desirable, inasmuch as its various properties doubtless depend in a large part upon the proportion of these three components of the leaf—namely, soft tissue, vein, and grain.

CHEMICAL NATURE OF GRAIN

Chemically, the grain has been supposed by Sturgis (7) and by Loew (5, p. 38–39) to be calcium oxalate, while from the fact that the grain particles produce minute explosions as fire reaches them in the process of

smoking, they have been considered by many to be composed of potassium nitrate. However, it has been shown that the grain is slowly soluble in water. This was done by allowing free-hand sections of a fermented leaf to remain in water for 4 hours and also by permitting a piece of a similar leaf to soak in water on a slide for 17½ hours. In order to show the position of the grain bodies and the idioblasts containing calcium oxalate, camera-lucida sketches were made with the aid of polarized light at the beginning of the treatment with water. In both cases, at the end of 4 and 17½ hours, respectively, no crystalline material remained in the positions of the grain bodies, where at the beginning of the experiment the bodies were highly active in polarized light. In the positions of the bodies, however, both in the sections and in the piece of leaf, the cells that had contained the crystalline material were found to be markedly distended, although upon the application of the slightest pressure their walls immediately collapsed, giving additional proof that the substances which had formerly filled the cells and held them rigid had been dissolved out. The calcium oxalate sand crystals and the scattered, single crystals referred to above remained unchanged.

In a subsequent experiment a piece of fermented leaf tobacco was dehydrated in alcohol and cleared and examined in xylol to determine the character and distribution of the grain bodies and at the same time the position and abundance of the idioblasts and the single, scattered crystals. The xylol was then removed from the piece with alcohol and the latter in turn displaced by distilled water, in a relatively large volume of which the material was allowed to remain for 24 hours at room temperature. An examination with polarized light was then made with the specimen mounted in water. In this case also it was noted that the crystalline material had disappeared from the grain bodies, but it was also evident that neither the calcium oxalate sand crystals nor the single, scattered crystals had been affected by the treatment. This was verified by dehydrating, clearing, and mounting the tissue as already described. The piece of leaf was then run back through water and allowed to stand in 50 per cent acetic acid for 48 hours and mounted and examined in the same medium. Both at this examination and after washing in water and again dehydrating and clearing, no scattered, single crystals could be found—that is to say, the only crystalline material remaining in the tissue after successive treatments with distilled water and with 50 per cent acetic acid was the sand crystals of calcium oxalate. However, these last all disappeared, leaving no optically active crystalline substance whatever in the cells after the tissue had been subjected to treatment with 50 per cent hydrochloric acid for about 17 hours.

When separated from the surrounding tissues, however, the grain bodies seemed to be less readily soluble in distilled water than when they were treated *in situ*. This is probably due to the presence of some substance in the tissues of the leaf which affects the solubility of the grain. In

every case, however, the grain-forming material was dissolved upon treatment with water for 24 hours. There were left in the cells which had formerly contained the solid crystalline substance the scattered, single crystals and also frequently calcium oxalate sand crystals an idioblast of which had been included in the group of cells which became petrified through the deposit in them of the grain-forming substance.

HYGROSCOPIC PROPERTIES

In connection with the physical and chemical characteristics of the grain may also be mentioned the results of some determinations of the hygroscopicity of some of the component parts of the leaf procured by the mechanical process already described. For these determinations four classes of air-dry material were used: (1) the leaf web—that is, the soft, parenchymatous tissue which passed through a 200-mesh sieve; (2) large veins—that is, those which, excluding the midrib, would not pass through a 20-mesh sieve; (3) smaller veins, which passed through a 30—but not an 80-mesh sieve; and (4) grain, a mixture of various sizes. Two-gm. samples of each of these classes were exposed for 50 hours side by side in open, tared Petri dishes to an atmosphere containing moisture derived from 125 gm. of granulated cigar-filler tobacco containing 26 per cent of moisture, a tight desiccator kept at about 30° C. being used as a container. The percentages of moisture absorbed by the samples were determined in the usual way, with the following results: Leaf web, 20 per cent; large vein, 17 per cent; small vein, 25 per cent; and grain, 14 per cent.

The granulated tobacco was used as a source of moisture in order to produce, as nearly as possible, natural conditions and the greatest competition between the samples and also between these and the source of moisture, at the same time insuring against the condensation of moisture within the desiccator through changes in temperature. While these results are products of work of a preliminary nature, enough has been done to indicate that the grain is the least and the small veins the most hygroscopic of the kinds of material studied. In connection with the latter it is believed that their power to absorb so large an amount of moisture may be due, at least in part, to a colloidal substance present in some of their xylem or phloem elements. This substance has the property of swelling markedly and protruding from the ends of pieces of small veins when the latter are submerged in water.

QUALITATIVE TESTS

Qualitative tests of the pure grain and its ash showed an abundance of calcium, some potash and magnesia, and a little ammonia, but only traces of oxalic, nitric, and sulphuric acids, the last of which was proved to arise chiefly from the combustion of proteids in the process of ashing.

The ash of the grain, however, contained a large amount of carbonate and therefore indicated the presence of some organic acids other than oxalic.

Aside from the solubility of the grain material in water and the detection of only a small amount of oxalic acid by ordinary qualitative means, Borodin's method (2), which consists of treating the substance to be tested with a saturated solution of the suspected substance, showed the grain to be composed of a salt or salts other than calcium oxalate.

Further, through the interest and kind assistance of Dr. F. E. Wright, of the Geophysical Laboratory of the Carnegie Institution of Washington,[1] the application of the petrographic microscope and methods to a study of the crystalline substances of the grain revealed the presence of normal calcium malate in these bodies. These methods also showed the presence of other crystalline substances, the identity of which has not yet been established. As is evident from the detailed quantitative analyses hereinafter recorded, the grain is a mixture of salts some of which may be double or even triple combinations, and the difficulties of procuring these various possible compounds in crystalline form for a comparison of their optical properties with those of the salts entering into the composition of the grain have not been surmounted at this stage of the investigation.

QUANTITATIVE ANALYSES

The data given in Table I represent quantitative determinations made on portions of the same material as that from which samples were taken for a study of the hygroscopic properties. The mineral components were determined by the official methods [2] and the organic acids by the method of Kissling.[3]

TABLE I.—*Composition of the leaf web, vein, and grain of Pennsylvania tobacco, on basis of sand-free air-dry material*

Constituents determined.	Leaf web passing through 200-mesh sieve.	Large vein (excluding midrib) not passing through 20-mesh sieve.	Small vein passing through 30-mesh but not 80-mesh sieve.	Grain of various sizes.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Moisture	7.80	7.11	6.25	8.06
Pure ash	16.48	17.80	16.91	40.26
Potassium oxid	4.11	5.72	5.29	3.42
Calcium oxid	6.70	6.65	6.6	26.34
Magnesium oxid	1.33	1.46	1.59	3.13
Oxalic acid	3.16	2.75	3.0	.82
Citric acid	3.26	1.15	2.91	22.38
Malic acid	3.46	4.09	5.24	13.58
Total determined[a]	29.82	28.93	30.88	77.73

[1] The writer wishes to express his deep appreciation of Dr. Wright's assistance.

[2] Wiley, H. W., ed. Official and provisional methods of analysis, Association of Official Agricultural Chemists. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig. 1908. Reprinted, 1912.

[3] Kissling, Richard. Beiträge zur Chemie des Tabaks. Zur Tabakanalyse. *In* Chem. Ztg., Jahrg. 28, No. 66, p. 775–776, 1 fig. 1904.

[a] Pure ash not included.

The analyses recorded in Table I show that the grain contains a very high percentage of ash, that calcium is by far the most abundant constituent, that nearly as much magnesium is present as is contained in the three other parts of the leaf taken together, but that a smaller amount of potash is found in the bodies than in any other class of leaf material studied. Of the organic acids, citric and malic are abundant in the grain, while oxalic acid is present in a much smaller percentage than in any of the other classes of material. These data indicate that the grain material must be a mixture of citrates and malates, chiefly of calcium, with some magnesium and a little potassium. Nicotin seems to enter into the composition of the grain also, since about 1.5 per cent of that substance was found in an analysis of one sample of grain. The totals of the percentages of substances determined indicate that the grain (77.73 per cent total determined) contains little material other than these substances. There remains in this case only about 22 per cent undetermined, and this must account for cellulose, nicotin, and other nitrogenous compounds, sulphur compounds, and phosphoric acid, as well as small quantities of sodium, iron, aluminum, etc., while in the three other classes these undetermined substances constitute in each about 70 per cent.

CORRELATION OF THE GRAIN WITH BURNING QUALITY

Although persons experienced in the handling of tobacco consider that a well-developed grain is an indication of good quality, particularly with reference to the burn, it seems that this is a matter of practical experience with them rather than a factor permitting definite discussion or explanation.[1] They assert that a "close-grained" leaf will burn poorly, while one possessing an "open grain" will have a greater fire-holding capacity. Recently, in the course of interviews with packers and manufacturers in Lancaster County, Pa., these observations have been found capable of substantiation by microscopic examination. The writer requested each of several practical tobacco men to select, on the character of the grain alone, what he considered to be a good-burning and a poor-burning leaf. In making this selection the leaf was stretched and while taut was allowed to pass slowly through the fingers, usually accompanied by the remark that the leaf showed an open grain or that it was close-grained. The leaves designated as poor-burning (close-grained), invariably possessed a hard texture—that is, something of the nature of the softer grades of paper—always showed poor elasticity, and rarely exhibited grain bodies on the surface. On the other hand, the leaves judged to be good-burning (showing an open grain) were comparatively soft in texture, elastic, and usually possessed grain bodies sufficiently large to

[1] See also Hayes, H. K., East, E. M., and Beinhart, E. G. (4, p. 28). The authors used seven classes to indicate the prominence of grain in considering the quality of the strains studied.

be visible to the unaided eye. It may be mentioned, however, that occasionally a leaf was found which was classed among the "open-grained" the grain of which was not apparent on the surface of the leaf, though evidently the experienced hand could detect its presence and determine its character.

The burn of the leaves thus selected was tested, always with the result predicted. A small piece was then cut from the unburned portion of each leaf at about a quarter of an inch from the point at which the fire was extinguished. These pieces were dehydrated in absolute alcohol, cleared and mounted in cedar oil, and examined microscopically with polarized light. Without exception, the good-burning leaf showed that the grain material had become well aggregated into definite bodies separated one from the other by a band of tissue free from crystalline material, save for the scattered, single crystals and occasional idioblasts containing calcium oxalate. This condition was evident even in the open-grained leaves mentioned above in which no grain was macroscopically visible on the leaf, for in those cases the bodies, though too small to cause a swelling on the surface, were definite in form and sufficiently separated to allow a zone of comparatively empty cells around each.

In the pieces from the poor-burning (close-grained) tobacco, on the contrary, the crystalline grain material proved to be scattered more or less evenly throughout the tissue, without any considerable degree of aggregation into definite bodies large enough to leave an encircling zone of empty cells around each particle. A few poor-burning samples have been found, however, in which some aggregation of grain substance had occurred, but in these the intervening tissue was filled with a mass of grain material which, for some reason, had failed to form definite bodies, thereby producing the same condition found in the poor-burning samples which were without appreciable aggregation.

From these facts it would seem that a certain degree of aggregation, with intervening tissue free from grain substance, is necessary in order that good fire-holding capacity may be assured. This suggests that the substance composing the grain bodies may have in reality a retarding effect upon the advance of fire in the tissue and that zones of grain-free cells must be present in order that a sufficient degree of heat may be generated, by their more rapid combustion, to ignite the solid grain particles. Verification of this suggestion has partially been obtained by watching through a binocular microscope the progress of the fire in the tissue. In a piece of leaf with prominent grain it was found that in the process of burning the fire line passed part way around and in some cases even beyond the centers of large grain bodies before they became ignited. It seems reasonable to suppose that the same process takes place in a leaf in which the grain particles are too small to admit of investigation in this manner and, further, that in a close-grained leaf there

is not enough grain-free tissue to act as kindling material and produce the temperature required to ignite the almost solid mass of grain substance characteristic of poor-burning tobacco.

While several theories have been set forth[1] concerning the burn of tobacco, for the most part chemical and differing from the hypothesis just advanced, an instance in favor of the present explanation is found in the case of the notoriously poor-burning 1909 crop of Pennsylvania. In this tobacco the grain substance is abundant, but it occurs in the tissue as an almost solid sheet, showing very poor aggregation into definite grain bodies (Pl. 17, fig. B). Again, flue-cured tobacco, which is always considered a poor-burning type, is entirely without grain aggregates. In this tobacco there is a small amount of grain substance (Pl. 17, fig. C) compared with cigar types, and it appears to be deposited very rapidly in the form of a haze of minute crystals throughout the tissues of the leaf. It is thought that the rapid-curing method employed with this type is responsible for this condition, inasmuch as the same kind of tobacco, grown in the same region and air-cured instead of being subjected to heat, shows to some degree the aggregation of the grain substance into definite bodies. In this connection it may be said also that Connecticut tobacco (Pl. 17, fig. D), which as a rule shows an excellent burn, also possesses a high degree of aggregation of the grain substance.

In connection with some investigations concerning the cause of the defects in the burning quality of York County tobaccos jointly pursued by the Office of Tobacco and Plant Nutrition Investigations and the Pennsylvania Agricultural Experiment Station[2] working cooperatively, a certain degree of correlation of grain formation and burning quality has been found. The tobacco was grown at Red Lion, Pa., during the seasons of 1913 and 1914 in duplicate plots upon which nine different fertilizer treatments, identical for the two years, were used to determine their influence upon the burning quality of the product. The material used for microscopical investigations was comparable to that upon which the burn and other factors were determined. In preparing the material for examination with the microscope, disks were cut from the leaves

[1] Garner discusses (3) the more important contributions concerning the burn of tobacco. Of the two physical theories thus far advanced, the one sought (1) to correlate the burning quality of tobacco, at least in part, with such anatomical features of the leaf as the number of rows of cells, the size of intercellular spaces, etc., while the other attributes (6) a beneficial influence to the action of potash salts of the organic acids in swelling to many times their original bulk and thereby yielding a porous mass of finely divided carbon when decomposed by heat. The chemical data given herewith do not support the latter theory, since the organic acids are largely localized in the grain, and only a small portion of the potassium is present in these bodies. This indicates that the greater portion of the potassium in the leaf is not in combination with the organic acids. No experimental work has been done by the writer concerning the former theory, though it is believed that in view of the results published herein and the fact that in cigar tobaccos the intercellular spaces are obliterated and the cell walls collapsed during the fermentation process little ground would be found for its substantiation.

[2] A report of this cooperative work by Dr. William Frear, Chemist and Vice Director of the Pennsylvania Agricultural Experiment Station, will appear in the annual reports of that institution for 1913–14 and 1914–15.

after fermentation and these were dehydrated in absolute alcohol, cleared in xylol, and mounted in Canada balsam.[1]

In ordinary transmitted light the samples showed no marked variations one from the other. The use of polarized light, however, revealed striking differences in the degree to which the grain substance had become aggregated, and it was found that these differences seemed to remain fairly constant between the good-burning samples on the one hand and the poor-burning samples on the other. In order that the condition found might be expressed in some form more concise than by descriptive terms, a system of scoring the degree of aggregation was adopted. By using a maximum perfect score of 10 points, convenient minor classes occurred as follows: "Good," 8 points; "fair," 6 points; "poor," 4 points; and "very poor," 2 points. A detailed score (Table II) of a poor-burning sample will best serve to illustrate the method used.

TABLE II.—*Detailed score of poor-burning sample from Plot I, Red Lion, Pa., 1913*

Disk No.	Grain.	Score.
1	Poor aggregation; small amount; haze of single and cryptocrystalline crystals	4
2	Poor aggregation; small aggregates; flaky spherites, cryptocrystalline haze	3
3	Very poor aggregation; small amount; fine cryptocrystalline haze with larger singles	2
4	Very poor aggregation; dense, hazy mass	2
5	Poor aggregation; similar to disk 4, though less dense	3
6	No aggregation; spherites and cryptocrystalline haze	1
7	Very poor aggregation; similar to disk 4	2
8	No aggregation; fine, thin cryptocrystalline haze	0.5
9	Poor aggregation; dense; a few flaky and a few black superficial aggregates	2.0
		19.5
	Divided by 9, score is	2.16

It will be noted in the score that the word "spherites" is used in the description of some of the samples. These are of much more frequent

[1] Of the 1913 material only the two best and the two poorest-burning samples were studied in detail, while the samples from all the fertilizer treatments used in 1914 were subjected to an equally thorough investigation. The microscopical data here presented represent, for the 1913 material, detailed examinations of disks cut from each of 18 representative leaves from each of the four fertilizer treatments after the fermentation process was completed. In 1914, however, the leaves were selected in the field before the plants were harvested. In this case three leaves from each of six average plants in each treatment were sampled in the field by removing a small disk of tissue from each leaf selected at a point equidistant from the midrib and the margin and midway between the base and apex. These disks were killed in absolute alcohol and preserved for future study in the same liquid. The leaves thus sampled were the eleventh, twelfth, and thirteenth below the point of topping, which point in all of the plants bore, as nearly as possible, the same relation to the developing flowers. These same leaves, having been tagged in the field, were used later in a preliminary study of the development of the grain and, after fermentation, for determining the character of the fully developed grain together with the fire-holding capacity and certain chemical analyses.

occurrence in poor-burning than in good-burning tobacco, as are also large, single crystals of grain substance and aggregates composed of large, platelike or flaky crystals. The cryptocrystalline haze is also much more prominent in the tissues between the relatively few definite grain bodies in the poor-burning tobacco. It will also be noted that no account is taken of the calcium oxalate or the single, scattered, acetic-acid-soluble crystals. The result of this method and the correlation of this result with apparently related factors are seen in Tables III and IV and figures 1 and 2. Corresponding data relative to the potash and chlorin content of the tobaccos are also included in the tables.

TABLE III.—*Data relative to the burning quality of tobacco from fertilizer treatments at Red Lion, Pa., crops of 1913 and 1914*

Treatment.	Burn score (maximum=20 points).[a]	Fire-holding capacity in cigar.[a]	Grain aggregation (Maximum=20 points. Original score × 2).[b]	Potash content.[a]	Chlorin content.[a]	Potash-chlorin ratio.[a]	Burning quality.
			1913				
		Min. sec.		*Per cent.*	*Per cent.*		
I	13.3	4 25	5.4	3.7	1.78	2.3	Poor.
III	18.0	5 37	13.2	4.01	.56	7.1	Good.
VII	17.5	6 7	12.8	4.14	.64	6.7	Do.
IX	11.0	4 22	7.3	3.44	2.66	1.3	Poor.
			1914				
I	14.0	4 10	5.7	3.61	1.38	2.6	Poor.
III	18.0	8 0	11.1	3.42	.26	13.15	Good.
VII	18.0	6 15	9.7	3.57	.23	15.5	Do.
IX	12.0	3 45	4.3	3.34	2.44	1.36	Poor.

[a] Data furnished from report of cooperative work by Dr. William Frear, Chemist and Vice Director of the Pennsylvania Agricultural Experiment Station. The burn score and fire-holding capacity in the cigar were determined by Mr. Otto Olson, Assistant in Tobacco Investigations, Bureau of Plant Industry, and the determinations of the potash and chlorin content and the potash-chlorin ratio were made by Mr. E. S. Erb, Assistant Chemist, Pennsylvania Agricultural Experiment Station.

[b] Data obtained by the writer and included in the report of Dr. W. W. Garner on the portion of the cooperative work undertaken by the Office of Tobacco Investigations, Bureau of Plant Industry.

In Tables III and IV and figures 1 and 2 the fire-holding capacity of the cigar has reference to the length of time a cigar made entirely of the tobacco in question remains lighted. The burn score includes the fire-holding capacity, amount of charring, character of ash, etc.

In determining the fire-holding capacity of the open leaf the writer ignited separately each leaf of the sample with the glowing end of a piece of punk, and the number of seconds during which the fire glowed in the larger portion of the burning area was taken as the fire-holding capacity.

TABLE IV.—*Data relative to the burning quality of the tobacco grown on fertilizer plots at Red Lion, Pa., in 1914*

Treatment.	Fertilizer applied per acre. Kind.	Quantity.	Burn score (maximum 20 points).[a]	Fire-holding capacity in cigar.[a]	Fire-holding capacity, punk-stick method.[a]	Grain aggregation score (maximum 20 points. Original score × 2.[a])	Potash content.[a]	Chlorin content.[a]	Potash-chlorin ratio.[a]
		Pounds.		*Min. sec.*	*Sec.*		*Per ct.*	*Per ct.*	
I	Manure	20,000	14.0	4 10	8.0	5.7	3.61	1.38	2.6
II	Manure	20,000	16.0	5 0	6.2	6.0	3.85	.91	4.2
	Dissolved rock	343							
	Potassium sulphate	100							
III	Cottonseed meal	1,485	18.0	8 0	16.8	11.1	3.42	.26	13.15
	Potassium sulphate	258							
IV	Cottonseed meal	1,485	17.5	5 22	14.8	7.1	3.17	.18	17.61
	Dissolved rock	190							
	Potassium sulphate	258							
V	Cottonseed meal	1,485	17.0	5 45	14.2	8.1	3.75	.19	19.73
	Dissolved rock	372							
	Potassium sulphate	258							
VI	Cottonseed meal	1,485	17.5	5 7	19.8	8.1	4.06	.23	17.7
	Potassium sulphate	129							
	Potassium carbonate	112							
VII	Cottonseed meal	1,485	18.0	6 15	20.5	9.7	3.57	.23	15.5
	Precipitated bone	136							
	Potassium sulphate	258							
VIII	Cottonseed meal	1,485	17.0	5 52	10.1	7.0	3.47	.23	15.08
	Potassium carbonate	224							
	Dissolved rock	190							
IX	Cottonseed meal	1,485	12.0	3 45	5.4	4.3	3.34	2.44	1.36
	Dissolved rock	372							
	Potassium chlorid	258							
Average for treatments I, II, IX			14.0	4 18	6.5	5.3	3.6	1.58	2.7
Average for treatments III to VIII, inclusive			12.5	6 35	16.0	8.5	3.57	0.22	16.46

[a] Same as for Table III.

As is illustrated by figures 1 and 2, representing the data included in Tables III and IV, respectively, the curves for the burn, the fire-holding capacity in the cigar, and the grain aggregation show a marked parallelism, although in the fire-holding capacity determined on single leaves the second highest point in the curve does not correspond to either of the principal maxima in the three other curves.

While the relative values representing the physical factors determined for the poor-burning samples correspond in a general way with Dr. Frear's

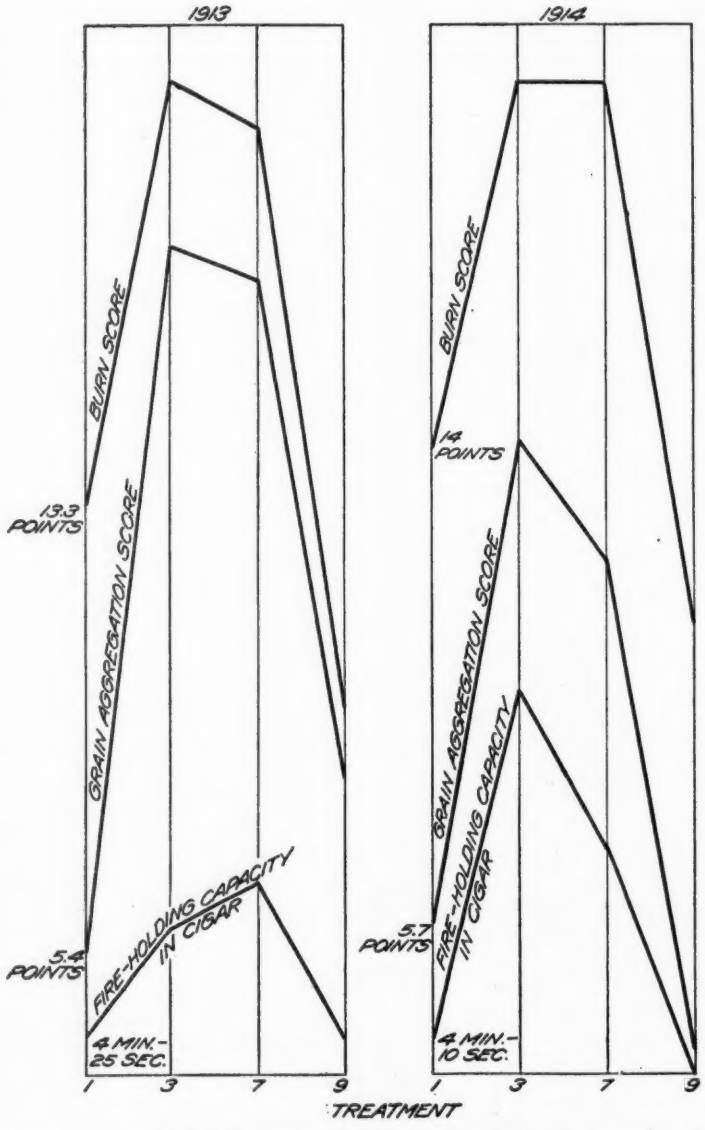


FIG. 1.—Curves plotting data relative to the burning quality of tobacco from fertilizer treatment at Red Lion, Pa., for crops of 1913 and 1914. Data taken from Table III.

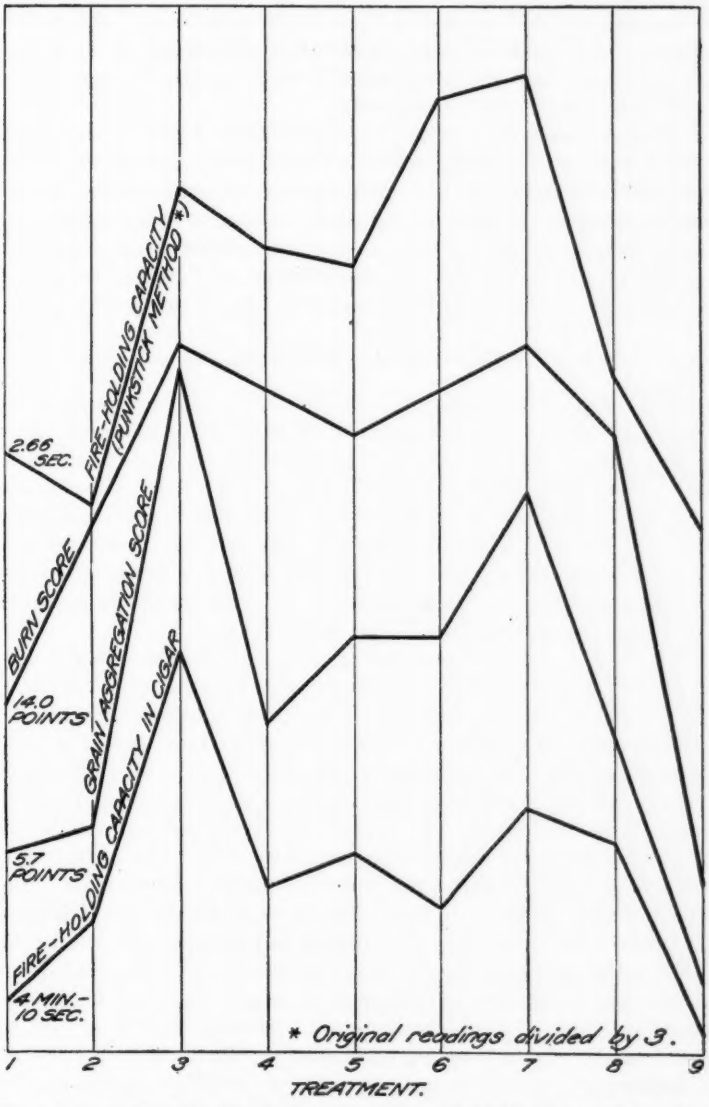


FIG. 2.—Curves plotting data relative to the burning quality of the tobacco grown on fertilizer plots at Red Lion, Pa., in 1914. Data taken from Table IV.

potash-chlorin ratios and show an inverse relation to his chlorin-content figures, this relation does not hold for the good-burning samples, except in the case of the Treatment VI previously referred to. The relative inferiority of the poor-burning samples in this series is doubtless traceable in part to their high content of chlorin, which element is commonly reputed to be antagonistic to combustion.

Though a more or less definite correlation seems to exist in the tobacco studied between the burning qualities and grain aggregation, for which a tentative explanation has been advanced, no reason is evident at this time for the apparent relation between the low degree of aggregation and the high chlorin content of the poor-burning samples. It is hoped that future work may throw some light upon this phase of the problem as well as upon other possible factors affecting grain formation in tobacco.

DEVELOPMENT OF THE GRAIN

While the opportunity has not been offered for a thorough study of the process of formation and aggregation of the grain in the leaf, preliminary investigations upon material referred to in footnote on page 279, as well as upon various kinds of tobacco observed in the laboratory, indicate that the grain substance is not present in crystalline form in the living, green leaf. These observations prove that the formation and development of the grain bodies are concomitant with the process of curing, and there are indications that the aggregation of the grain substance continues during fermentation (compare Pl. 17, fig. A, E, G).

At Red Lion, Pa., on September 22, 1914, four days after harvesting, samples of tobacco were becoming flecked near the margins of the leaves with the yellow mottling characteristic of the early stages of curing.

In this condition microscopic examination showed the absence of grain bodies and also that the protoplasmic contents of the cells were still intact. In the later stages of yellowing and with the apparent death and disorganization of the protoplasm, just before the development of the brown color, highly refractive droplets appeared in the cells. Upon attempting to dehydrate a piece of such a leaf in alcohol minute crystals appeared in these droplets. In the next stage—that is, at about the first indication of a brown color in the leaf—the droplets had enlarged and become decidedly viscid, and each showed upon examination with polarized direct sunlight conspicuous crossed extinction bands and presented the appearance of spherites. Upon dehydration at this stage they developed no small crystals. As the browning progressed, certain of the bodies seemed to differentiate within themselves, without artificial dehydration, a nucleus of crystals of definite though not identifiable form. The substance of the droplets in which no crystals had formed seemed to migrate toward those which had produced crystal nuclei, increasing by accretion the crystalline mass until groups of several cells each had become literally

petrified and formed a solid grain body. Definite grain was formed in leaves of average maturity in nine days after the tobacco was harvested.

It is thought that the migration of the still viscid substance was brought about by diffusion, through the dead and permeable protoplasm, set up by a lowering of concentration in the immediate vicinity of the developing crystal nuclei. Under ideal curing conditions it is probable that this process results in the aggregation of all, or nearly all, of the grain-forming substance into definite bodies, leaving around each a zone of cells largely free from that material. It is believed that under these conditions the number of grain bodies developed in a given area is dependent upon the number of crystal nuclei formed. The factors which determine the number and distribution of the latter are not known. It is thought, however, that the rate of desiccation and the degree of maturity of the curing leaves may be of importance in this respect.

The behavior of the grain material in the flue-cured tobacco seems to support the idea that the rate of drying is a factor in grain development. In the completely cured leaf of this type the substance which would have formed more or less definite grain bodies under ordinary air-curing conditions apparently has been thrown down in crystalline form by the rapid curing and drying characteristic of the method, in the first droplet stage referred to above, resulting in a haze of minutely crystalline material in all the cells. Even Connecticut tobacco, when cured very rapidly ("hayed-down"), frequently shows poor burning qualities. Again, in the poor-burning crop of tobacco produced in Pennsylvania in 1909, which was a "dry-weather crop," the grain material failed to become well aggregated. It seems probable that either a low percentage of water in the tissues or an abnormally large quantity of grain-forming substance in solution in the cell sap at the time of harvesting, or both, must have resulted in the formation of an unusually large number of crystal nuclei in a given area, especially if weather conditions were such as to cause rapid dessication in the early stages of curing. In the later stages, then, opportunity for the development of zones of cells free from grain substance would have been limited by the closeness together of the crystal nuclei. Indeed, samples of this crop have been seen in which the grain substance seemed to have been thrown down in practically every cell, much in the manner of that of flue-cured tobacco, though greater in amount. Of interest to note in passing is the fact that the burning properties of this crop, much of which is still in storage, are very gradually improving. It seems probable that this may be due in part to the slow aggregation of grain material rather than to the loss through aging of some substance injurious to the burning quality.

The leaves upon which the observations were made at Red Lion, Pa., were sampled after the curing had been completed, and it was found that the grain throughout the leaf was in practically the same condition

as had obtained near the outer edges of the leaves on the ninth day of curing. Examination after the same leaves had been fermented, however, showed that the grain was much more pronounced on the surface, and the microscope revealed a greater degree of aggregation than had existed at the end of the curing process. It is believed that with the high water content of the fermenting tissue the grain substance is gradually put in solution, particularly in the case of the smaller bodies, and that further grain aggregation and development of intervening zones of comparatively empty cells is thereby made possible.

SUMMARY

(1) The grain of cigar tobacco consists of hard bodies which, if sufficiently large, cause the surface of the leaf containing them to present a pimply appearance. This grain, in connection with other intimately related properties of the leaf, constitutes an important factor in determining the value of cigar tobacco.

(2) Each body consists of from one to several leaf cells distended with a mass of crystalline substance. They are most prominent microscopically when examined with polarized light. Although visible in ordinary transmitted light, owing to the concentration in them of a large part of the brown coloring matter of the leaf, the details of their structure are more apparent when the former method of examination is used.

(3) Based upon microscopic features, five forms or types of grain are recognized, though their significance is a matter still to be investigated.

(4) Two other kinds of crystalline material are found in the tobacco—namely, cryptocrystalline calcium oxalate, contained in certain cells in the various tissues of the leaf; and single, small, prismatic crystals scattered evenly throughout the leaf, one in nearly every cell of the mesophyll and epidermis.

(5) A mechanical method resulted in the separation of the grain bodies from the other portions of the leaf and gave an approximate mechanical analysis. This analysis showed roughly the percentages by weight of leaf web, veins, and grain.

(6) Chemical analyses proved that the grain is composed chiefly of calcium, with a little magnesium and potassium, in combination with citric and malic acids rather than with oxalic acid. One of the salts, normal calcium malate, was identified by petrographic methods. Determinations of the hygroscopic properties of the component parts of the leaf separated by mechanical means indicate that the grain is not responsible for the marked hygroscopic properties of tobacco, since it absorbed the least water from a moist atmosphere. The small veins showed the greatest hygroscopicity.

(7) The grain bodies of tobacco are developed in the course of postmortem changes which take place during the process of curing and con-

tinue during fermentation. A microscopically visible change consists of a more or less complete aggregation of the grain-forming substance of all the cells into certain groups of cells. The factors determining the location of these groups are unknown.

(8) In the tobacco studied a correlation was found between the grain and burning properties. It is believed that the substances contained in the grain bodies are injurious to the burn and that the quality of the latter is dependent upon the degree to which the former are aggregated into definite bodies sufficiently separated, one from the other, to permit a considerable fire-carrying zone of cells, emptied of grain material, around each. The influence of the degree of aggregation of the grain substance upon the color, texture, and elasticity of the leaf has not yet been thoroughly investigated.

LITERATURE CITED

(1) BEHRENS, Johannes.
1894. Weitere Beiträge zur Kenntnis der Tabakpflanze. V. Der anatomische Bau und die Bestandteile des Tabakblatts in ihrer Beziehung zur Brennbarkeit. *In* Landw. Vers. Stat., Bd. 43, p. 271–301.

(2) BORODIN, Johann.
1878. Ueber die physiologische Rolle und die Verbreitung des Asparagins im Pflanzenreiche. *In* Bot. Ztg., Jahrg. 36, No. 51, p. 801–816; No. 52, p. 817–831.

(3) GARNER, W. W.
1907. The relation of the composition of the leaf to the burning qualities of tobacco. U. S. Dept. Agr. Bur. Plant Indus. Bul. 105, 25 p.

(4) HAYES, H. K., EAST, E. M., and BEINHART, E. G.
1913. Tobacco breeding in Connecticut. Conn. Agr. Exp. Sta. Bul. 176, 68 p., 12 pl.

(5) LOEW, Oscar.
1900. Physiological studies of Connecticut leaf tobacco. U. S. Dept. Agr. Rpt. 65, 57 p.

(6) SCHLOESING.
1860. Chimie appliquée a la vegetation.—Nouvelles recherches sur le tabac. *In* Compt. Rend. Acad. Sci. [Paris], t. 50, no. 13, p. 642–644.

(7) STURGIS, W. C.
1900. On the so-called "grain" of wrapper tobacco. *In* Conn. Agr. Exp. Sta. 23d Ann. Rpt., 1899, p. 262–264, pl. 2.

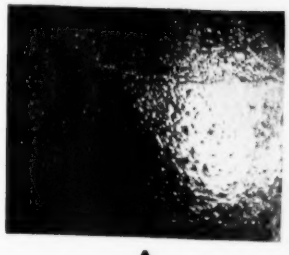
(8) TUNMANN, Otto.
1913. Pflanzenmikrochemie . . . 631 p., illus. Berlin.

PLATE 15

Fig. A, B.—Well-cased tobacco leaves stretched over the closed end of a test tube; showing very pronounced grain development. × 2.

Fig. C.—A portion of a cigar wrapped with a leaf containing very coarse grain. × 2.

Fig. D.—The same as figure C, but after a portion of the cigar had been smoked, showing the white pimples in the ash produced by the burning and swelling of the grain bodies. × 2.



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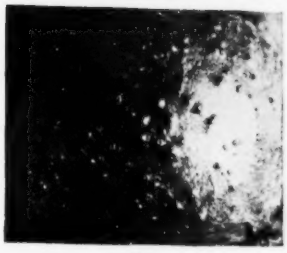

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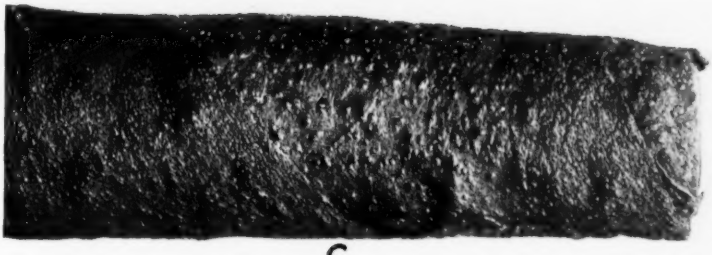

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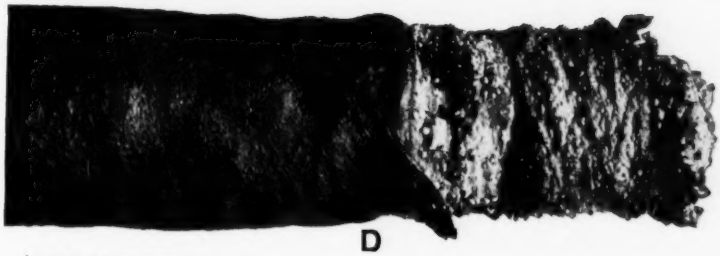

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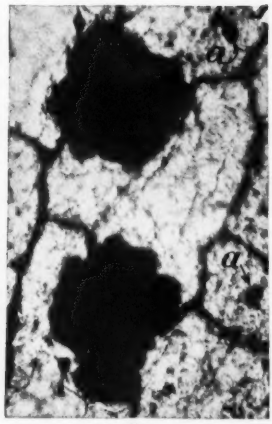

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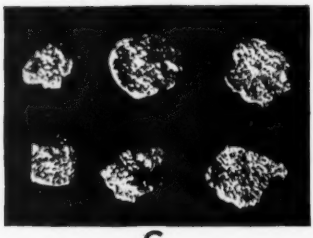

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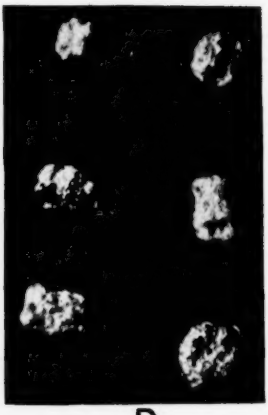

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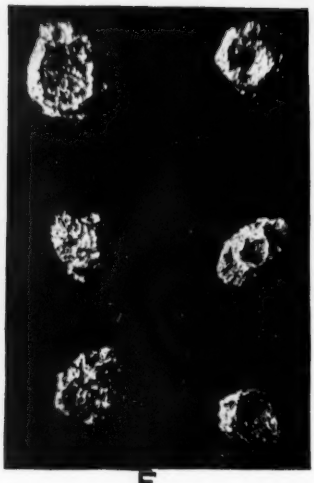

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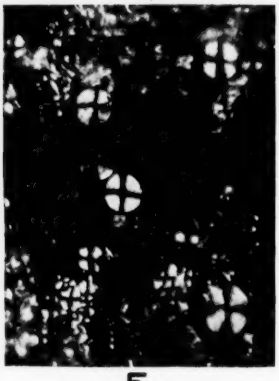

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PLATE 16

Fig. A.—Grain bodies of Connecticut Broadleaf tobacco as seen in ordinary transmitted light. *a*, Idioblasts containing sand crystals of calcium oxalate. × about 300.

Fig. B.—Representative grain bodies of class 1. × 19.5.

Fig. C.—Representative grain bodies of class 2. The high lights show the positions of the distended epidermal cells. × 19.5.

Fig. D.—Representative grain bodies of class 3. × 19.5.

Fig. E.—Representative grain bodies of class 4. × 19.5.

Fig. F.—Grain substance in the form of minute spherites. Photographed with polarized light. × 280.

PLATE 17

Fig. A.—Green tobacco leaf from Treatment I, Red Lion, Pa., 1914, killed in absolute alcohol and showing idioblasts of calcium oxalate and minute, scattered, single crystals of an undetermined substance, but no grain. Photographed with polarized light. × 65.5.

Fig. B.—Representative sample of the poor burning 1909 Pennsylvania tobacco. Photographed with polarized light. × 65.5.

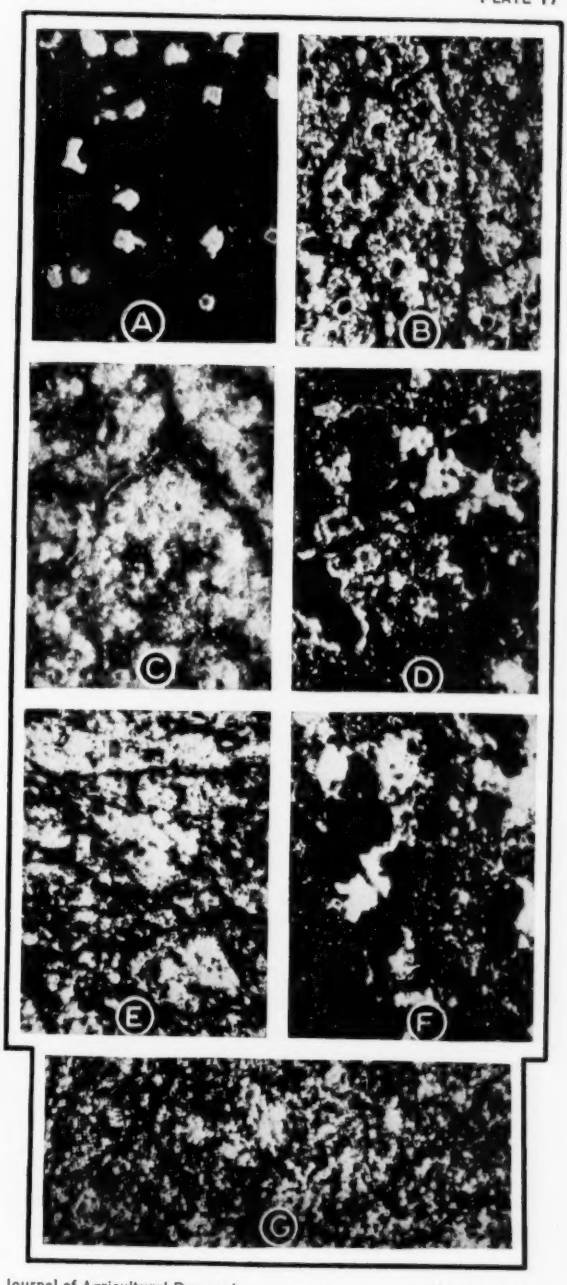
Fig. C.—Flue-cured tobacco. Poor-burning. Photographed with polarized light. × 65.5.

Fig. D.—Connecticut Broadleaf tobacco. Good burning. The brown coloring matter concentrated in the grain partially masks their structure in some cases. Photographed with polarized light. × 65.5.

Fig. E.—Fermented tobacco from Treatment I, Red Lion, Pa., 1914. Poor burning. Photographed with polarized light. × 65.5.

Fig. F.—Fermented tobacco from Treatment III, Red Lion, Pa., 1914. Good burning. The brown coloring matter concentrated in the grain partially masks their structure in some cases. Photographed with polarized light. × 65.5.

Fig. G.—Tobacco from Treatment I, Red Lion, Pa., 1914. Cured only. Photographed with polarized light. × 65.5.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HOST PLANTS OF THIELAVIA BASICOLA

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The increasing economic importance of *Thielavia basicola* Zopf as a root parasite of certain cultivated plants has led to a desire for more complete information regarding its range of host plants. This knowledge is important not only from the standpoint of the effect upon the hosts themselves but in the relation of the use of these host plants in rotation with other crops susceptible to attack by the fungus. *T. basicola* appears to be primarily a parasite of leguminous plants, and the common use of these in rotation may prove to be unprofitable for certain crops. The benefit derived from the added fertility in the soil may be entirely offset in certain instances by the injury done as a result of maintaining the fungus in the soil through the use of host plants in rotation.

From a mycological point of view, considerable interest may also be attached to the hosts of *T. basicola*. The earlier botanists who observed this fungus were in some doubt as to the parasitic nature of the organism. This view has persisted to some extent, and the fungus is still believed by some to be purely superficial in its mode of life and not ordinarily injurious when present. On the other hand, some of the later investigators have not only convinced themselves of the parasitism of the organism but also of a high degree of pathogenecity, while some have gone so far as to suppose the fungus capable of attacking almost any plant under favorable conditions (11).[2] It is fairly clear to the writer that this difference in opinion is due in a large measure to not only the species but the variety of host plant under observation. Considerable emphasis has also been laid upon the conditions necessary for disease to be developed by this fungus, leading to the supposition that certain unknown special conditions are necessary and that infection is difficult to obtain. Although this may be true within certain limits, it has been found that infection could be repeatedly and easily produced with the more susceptible plants. Where the writer has experienced difficulty in obtaining infection on certain host plants, it has been attributed rather to resistance or immunity in the

[1] This investigation was carried on in cooperation with the Bureau of Plant Industry, Department of Agriculture.

[2] Reference is made by number to "Literature cited," p. 299-300.

plant itself than to lack of favorable conditions for fungus attack. Plants of known susceptibility were at all times grown as controls in the infection experiments and served as a good basis for such conclusions.

HISTORICAL REVIEW

Berkeley and Broome (2, p. 461) in 1850 first described the fungus now known as *T. basicola*. These authors found it at the base of stems of *Pisum sativum* and *Nemophila auriculata*. The fungus was apparently not again noted until 1876, when Zopf (23) reported it from Germany on *Senecio elegans* and Sorokin (20) from Russia on horseradish (*Cochlearia armoracia*). Massee (10) described a new fungus in 1884 which he found on decaying leaves of *Blysmus compressus* and named it *Milowia nivea*. This fungus he considers in a later paper (11) as having been *T. basicola*. From descriptions and drawings of this earlier described form, however, it may be seen that there is some room for doubt as to the identity of these two forms.

In 1891 Zopf (24) again published upon the occurrence of *Thielavia basicola* and noted that it was especially common on leguminous plants, adding the following new hosts: *Lupinus angustifolius*, *L. albus*, *L. thermis*, *L. luteus*, *Trigonella coerulea*, and *Onobrychis crista-galli*. In the same year Thaxter (22) made the first report of the occurrence of *T. basicola* in America, finding it on the violet. Sorauer (19) in 1895 reported the fungus as causing a disease of the roots of the cyclamen. Peglion (13), working in Italy, in 1897 was first to record the parasitism of *T. basicola* on *Nicotiana tabacum*. Killebrew (8, p. 162), however, as early as 1884, described the symptoms of a root disease of tobacco in Pennsylvania, which was undoubtedly due to *T. basicola*, though no causal organism was named in the description. Selby (15, p. 228) has noted *T. basicola* as occurring upon the roots of *Begonia rubra*, following the nematode disease, and later found it causing a rootrot of *Catalpa speciosa* (17, p. 384, 447). Smith (18, p. 35–38) in 1899 added two new hosts, *Gossypium herbaceum* and *Vigna sinensis*. Van Hook (16, p. 96) was first to note *T. basicola* upon *Panax* (*Aralia*) *quinquefolium*. Aderhold (1) in 1905 found *T. basicola* on begonia and carried on infection experiments with pure cultures of the organism. He obtained slight infections on *Scorzonera hispanica*, *Daucos carota*, *Apium graveolens*, and *Beta vulgaris* and better development on *Lupinus angustifolius* and *Phaseolus vulgaris*. He concluded from his experiments that *T. basicola* was only a weak parasite.

Kirchner (9) includes two previously unreported species as hosts, *Phaseolus multiflorus* and *Nicotiana rustica*. Since no reference to personal observation or infection experiments could be found, it is presumable that these are not authentic host plants, especially in view of the facts noted later.

Gilbert (7) in 1909 added three new hosts: *Linaria canadensis*, *Oxalis corniculata*, var. *stricta*, and *Trifolium repens*. Chittenden (5) in 1912 reported *T. basicola* as a parasite of sweet peas. Massee (11) in the same year added to the list of host plants a species of Cypridpedium, *Aster* spp., and *Capsella bursa-pastoris*.

Rosenbaum (14) published in 1912 on infection experiments with *T. basicola*, and by cross-inoculation experiments found that the species of Thielavia on tobacco, cotton, and ginseng were identical.

O'Gara (12) in 1915 found *T. basicola* causing a disease of *Citrullus vulgaris* in Utah. This is the first report of the fungus on a member of the cucurbit family. Recently Burkholder (3) has noted the fungus on *Trifolium pratense*, *Trifolium hybridum*, and *Medicago sativa*.

METHOD OF WORK

The investigation of the host plants of *T. basicola* was undertaken primarily to corroborate, so far as possible, the hosts reported by earlier investigators, as in the majority of instances this had not been done. As it was deemed important to know something of the possible relation of the fungus to our agricultural plants, these were for the most part included in the earlier tests; but later the trials were made to include as many species as obtainable of the more susceptible families of plants. In this way about 200 species of plants have been grown on infected soil. The work was carried on almost entirely in the greenhouse. The seeds or plants were sown or transplanted into the infected soil, and optimum conditions maintained, so far as possible, especially as regarded the moisture content of the soil. The work was done partly at Arlington, Va., in cooperation with the Office of Tobacco Investigations, Bureau of Plant Industry, and partly at the Wisconsin Agricultural Experiment Station at Madison. In the Arlington greenhouses a fairly heavy clay loam soil from a tobacco field on the farm was used. This soil was taken from a spot in the field known to be badly infected with the rootrot caused by *T. basicola*. At Madison a greenhouse soil containing considerable vegetable matter was infected with soil from a tobacco field, where this rootrot had been occurring annually in recent years. On the whole, the conditions were such as might occur outdoors during a season of high precipitation. Recording soil and air thermometers showed temperatures ranging ordinarily between 18° and 25° C. in the soil and 20° to 30° C. in the air. The data were taken largely from infection on seedlings, as this was naturally an advantage in examining minutely the root system of a large number of plants. Since the age of the individual roots is considered a greater factor than that of the entire plant in determining the occurrence of infection, it is believed the results would be comparable if older plants were used.

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At intervals of about five days after the plants were well above the ground one or more plants were carefully removed from the soil with as much root system as possible. The soil was then washed off the roots, after which they were usually placed immediately in 80 per cent alcohol and transferred to the laboratory, where microscopical examination of any spots or lesions occurring on the roots were made. Where *T. basicola* was found without difficulty, three to five examinations only were made; but where it could not be found on reported or suspected hosts, the roots of 10 to 15 plants were carefully examined, except in a few rare instances where this number of plants could not be obtained.

EXPERIMENTAL RESULTS

The results obtained are presented largely in the following tables. The host plants are separated into those reported by earlier workers (Table I) and the new hosts (Table II). For convenience in reference the authority for the first report and the country and year in which reported are given. These columns are followed by the results obtained with the various plants in the present experiments, giving at the same time the approximate degree of susceptibility, as nearly as could be determined by the amount of infection obtained. In the same way these results are given for the new hosts reported here. From Table I it may be seen that out of the 39 host plants previously reported the parasitism of *T. basicola* on 25 of these has been corroborated. Of the remaining 14 species infection could not be obtained on 7 species. Seeds or plants of 7 species have not been secured up to this time, but infection in most of these cases was obtained upon closely related species, indicating at least that the unobtainable species are for the most part probably susceptible to attack. Sixty-six new species of plants have been added to those already reported and corroborated as host plants of *T. basicola*. As will be seen from the list, these are largely in the leguminous, solanaceous, and cucurbitaceous families. Although the plants tested were largely representatives of these families, a number of species of other families have been included in the tests, especially species of the Compositae, Gramineae, and Rosaceae. It is fairly safe to conclude from these tests that the latter families are generally immune from attack by *T. basicola*.

TABLE I.—*Host plants of Thielavia basicola reported before the present investigation*

Host plant.	Authority.	Locality.	Year.	Susceptibility.
1. Leguminosae:				
Pisum sativum	Berkeley and Broome (2).	England	1850	Low.
Onobrychis cristagalli.	Zopf (24)	Germany	1891	Medium.
Trigonella coerulea	do	do	1891	Do.
Lupinus angustifolius.	do	do	1891	Plants not obtained.
Lupinus thermis	do	do	1891	Do.
Lupinus albus	do	do	1891	Slight.
Lupinus luteus	do	do	1891	Medium.
Vigna sinensis	Smith (18)	United States	1899	Do.
Phaseolus vulgaris	Aderhold (1)	Germany	1905	Low.
Phaseolus multiflorus	Kirchner (9)	do	1906	None.
Trifolium repens	Gilbert (7)	United States	1909	Low.
Lathyrus odoratus	Chittenden (4)	England	1911	Slight.
Medicago sativa	Burkholder (3)	United States	1916	Low.
Trifolium pratense	do	do	1916	Do.
Trifolium hybridum	do	do	1916	Do.
2. Solanaceae:				
Nicotiana tabacum	Peglion (13)	Italy	1897	High; low.
Nicotiana rustica	Kirchner (9)	Germany	1906	None.
3. Cucurbitaceae:				
Citrullus vulgaris	O'Gara (12)	United States	1915	Low.
4. Miscellaneous families:				
Viola odorata	Thaxter (22)	do	1891	High.
Aralia quinquefolia	Van Hook (16)	do	1904	Medium.
Gossypium herbaceum	Smith (18)	do	1899	Do.
Nemophila auriculata	Berkeley and Broome (2).	England	1850	Plants not obtained.
Linaria canadensis	Gilbert (7)	United States	1909	Low.
Begonia rubra	Selby (15)	do	1896	Plants not obtained.
Begonia (tuberhybrida?).	Aderhold (1)	Germany	1905	Do.
Oxalis corniculata, var. *stricta.*	Gilbert (7)	United States	1909	Slight.
Catalpa speciosa	Selby (17)	do	1910	Do.
Senecio elegans	Zopf (23)	Germany	1876	Medium.
Aster sp.	Massee (11)	England	1912	Low.
Scorzonera hispanica	Aderhold (1)	Germany	1905	None.
Pastinica sativa	Taubenhaus (21)	United States	1914	Do.
Cochlearia armoracia	Sorokin (20)	Russia	1876	Slight.
Capsella bursa-pastoris.	Massee (11)	England	1912	Do.
Cypripedium sp.	do	do	1912	Plants not obtained.
Cyclamen sp.	Sorauer (19)	Germany	1895	Slight.
Blysmus compressus	Massee (11)	England	1912	Plants not obtained.
Apium graveolens	Aderhold (1)	Germany	1905	None.
Daucus carota	do	do	1905	Do.
Beta vulgaris	do	do	1905	Do.

TABLE II.—*New host plants of Thielavia basicola*

Host plant.	Susceptibility.
1. Leguminosae:	
Arachis hypogaea	Medium.
Astragalus sinicus	Do.
Cassia chamaecrista	Do.
Cytisus scoparius	Low.
Desmodium tortuosum	Slight.
Dolichos lablab	Do.
Galactia sp.	Low.
Glycine hispida	Do.
Lens esculenta	Medium.
Lespedeza striata	Low.
Lotus corniculatus	Do.
Lotus villosus	Do.
Lupinus hirsutus	Medium.
Medicago denticulata	Low.
Melilotus alba	Do.
Melilotus indica	Medium.
Ornithopsus sativus	Low.
Onobrychis viciaefolia	Slight.
Phaseolus acutifolius	Medium.
Robinia pseudoacacia	Do.
Sclotis chinensis	Low.
Strophostyles helvola	Medium.
Trifolium incarnatum	Do.
Trigonella foenum-graecum	Low.
Tephrosia virginiana	Do.
Ulex europaeus	Slight.
Vicia villosa	Do.
1. Leguminosae—Continued:	
Vicia faba	Slight.
2. Solanaceae:	
Datura metel	Medium.
Datura stramonium	Low.
Datura tatula	Slight.
Datura cornucopia	Do.
Nicotiana glauca (Gratz)	Low.
Nicotiana silvestris (Speg.)	Medium.
Nicotiana sanderae (Sander.)	Low.
Nicotiana repanda (Willd.)	Do.
Nicotiana atropurpurea	High.
Nicotiana langsdorffii (Weinm.)	Low.
Nicotiana chinensis (Fisch)	High.
Nicotiana macrophylla (Lehm.)	Low.
Nicotiana glutinosa (Linn.)	High.
Nicotiana calyciflora[a]	Medium.
Nicotiana latterrima (Mill.)	High.
Nicotiana alta (Link and Otto).	Medium.
Nicotiana angustifolia (R. and P.)	Do.
Nicotiana longiflora (Cav.)	Low.
Petunia (*hybridia*?)	Slight.
Solanum carolinense	Do.
3. Cucurbitaceae:	
Cucurbita maxima	Low.
Cucurbita pepo	Do.
Cucumis melo	Do.
Cucumis sativus	Slight.
Cucurbita moschata	Low.
Cucumis acutangulus	Do.
Cucumis flexuosus	Do.
4. Miscellaneous families:	
Viola tricolor	Slight.
Begonia semperflorens	Do.
4. Miscellaneous families—Continued:	
Nemophilia aurita	Medium.
Nemophilia insignis	Low.
Linaria cymbalaria	Do.
Linaria maroccana	Slight.
Paphiopedilum grossianum	Do.
Portulaca oleracea	Low.
Ipomoea coccinea	Slight.
Phlox drummondii	Do.
Papaver nudicaule	Do.

[a] The validity of these species may be questioned.

Negative results have also been obtained with a number of species in the most susceptible families. In the legume family these apparently immune species may be represented by *Phaseolus multiflorus*, *Vicia sativa*, and *Hedysarum coronarium*. In the Solanaceae there are varieties at least of *Capsicum annuum*, *Solanum melongena*, *Solanum tuberosum*, *Hyoscyamus niger*, and certain species of Nicotiana which are apparently immune to attack. *Benincasa cerifera* is the only species of the cucurbit family tested, which appears to be entirely immune from infection by *Thielavia basicola* when grown in infected soil. The common agricultural plants upon which infection could not be obtained were principally the

cereals—wheat (*Triticum* spp.), oats (*Avena sativa*), barley (*Hordeum* spp.), and rye (*Secale cereale*)—corn (*Zea mays*), potatoes (*Solanum tuberosum*), hemp (*Cannabis sativa*), flax (*Linum usitatissimum*), and sweet potatoes (*Ipomoea batatas*). With the vegetables the cabbage (*Brassica oleracea*), onion (*Allium cepa*), parsnip (*Pastinaca sativa*), carrot (*Daucus carota*), beet (*Beta vulgaris*), lettuce (*Lactuca sativa*), eggplant (*Solanum melongena*), and peppers (*Capsicum annuum*) appeared to be free from attack by the fungus; and of the fruits no infection could be obtained on strawberries (*Fragaria* spp.), raspberries (*Rubus* spp.), or blackberries (*Rubus* spp.).

Infection could not be obtained upon the following plants, which were previously recorded by others as being attacked by *T. basicola: Nicotiana rustica, Phaseolus multiflorus, Pastinica sativa, Scorzonera hispanica, Daucus carota, Apium graveolens,* and *Beta vulgaris.* The failure to obtain infection on these hosts may be attributed to one or more of several obscure causes. The writer is inclined to believe, however, that these species which have been mentioned as hosts of *T. basicola* should not be included in the list of host plants. There is a small probability of immune varieties or strains being used in the studies reported here. In an attempt to clear up this point different varieties of some of these species were used, but only negative results in infection were obtained.

Phaseolus multiflorus and *Nicotiana rustica* are included as hosts of *T. basicola* by Kirchner (9). A search through the literature has failed to reveal earlier reports of these species as hosts. It is evident that Kirchner included these species because of their relation to known susceptible species, as no mention of any original observations of his own could be found. This conclusion is further substantiated by the fact that after repeated tests by the writer, these species appear to be immune to attack.

Aderhold obtained very slight infections on *Scorzonera hispanica, Daucus carota, Apium graveolens,* and *Beta vulgaris.* His results were obtained, however, under quite artificial conditions and no doubt resulted from one form or other of injury to the roots. The conclusion was drawn that *T. basicola* was only a very weak parasite. All attempts to infect the above-named species, under the conditions of these experiments, show that these forms are immune or at least extremely resistant to attack by *T. basicola.* Aderhold's conclusion (1, p. 465)—

> Der Verlauf der Erscheinung zeigte, dass der Pilz kein heftiger Parasit ist. Ich schliesse mich auf Grund dieser Versuche der Auffassung Sorauers an, der Zufolge besondere Verhältnisse geboten sein müssen, um ihn zu einen wirklichen Schädiger zu machen—

should, therefore, certainly not be taken to apply to other host plants of *T. basicola.*

Massee (11) includes *Blysmus compressus* as a host of *T. basicola*, apparently on the basis of an early report (10) of *Milowia nivea* on decaying leaves of *B. compressus*. Unless this observation was corroborated by later observation, the writer believes some doubt may be entertained in regard to this species as a host, on account of the point of attack and question of the identity of this fungus with *T. basicola* Zopf. The description and figures in Massee's first article bear only a slight resemblance to *T. basicola*. Unfortunately the writer has been unable to obtain this species of Blysmus for trial in these experiments.

It is also interesting to note that certain species commonly cited as hosts of *T. basicola* are strikingly resistant or practically immune. Chittenden (4–5), Massee (11), and Taubenhaus (21) report *T. basicola* as a serious disease of sweet peas (*Lathyrus odoratus*), though they differ in their opinions as to the symptoms of the disease. Infection of sweet peas in the writer's experiments was obtained only with great difficulty and then only slightly. According to Taubenhaus, the organisms causing this infection of sweet peas and tobacco are interchangeable and no physiological race difference exists in the fungus. It seems plausible, therefore, that more resistant varieties of *L. odoratus* were used in the present trials than were used by Chittenden and Taubenhaus. It may be, therefore, that the sweet-pea disease may be controlled by selection for disease resistance. Taubenhaus also mentioned obtaining a culture of *T. basicola* from parsnip, although he does not include it in his list of hosts. No infection could be obtained upon that vegetable in the present trials.

Aderhold (1) failed to get infection on *Begonia semperflorens*, and it was only after repeated examinations that the writer found the fungus on this host and then only on nematode galls, as reported by Selby for *Begonia rubra*. *Cochlearia armoracia*, *Cyclamen* spp., *Pisum sativum*, *Lupinus albus*, *Catalpa speciosa*, and certain species of orchids were found to be very difficult to infect.

Plants upon which very slight infections were secured in one or two instances but are not included in the new list as hosts are *Lycopersicon esculentum*, *Tropaeolum majus*, *Fagopyrum esculentum*, and *Solanum nigrum*.

As a result of these studies, it is concluded, therefore, that certain members of the following families of plants are likely to be attacked by *Thielavia basicola* under favorable conditions for the development of the fungus: Araliaceae, Bignoniaceae, Compositae, Convolvulaceae, Crucifereae, Cucurbitaceae, Hydrophyllaceae, Leguminosae, Malvaceae, Orchidacae, Oxalidaceae, Papaveraceae, Polemoniaceae, Portulaceae, Primulaceae, Scrophulariaceae, Solanaceae, and Violaceae.

The occurrence of *T. basicola* on the various hosts studied differed principally in two respects: (1) The point of attack by the fungus and (2) the character of the sporulation. The occurrence of *T. basicola* in

certain cases on stems above the surface of the ground has been noted by others (11, 14), but is relatively rarely found. The lesions obtained on *Portulaca oleracea* were almost wholly on the low succulent stems, apparently irrespective of any infection at the base of the stem. Ordinarily, however, infection occurs only on the roots of the host plants or upon the base of the stem just at or below the surface of the soil (Pl. 18). In the case of *Nicotiana* spp., infection is ordinarily found on the secondary roots, although in some species the collar of the plants may be most markedly injured, whereas in the cucurbits the infection is usually on the stem just at or below the surface of the soil (Pl. 18, fig. A). In species of Pisum, Phaseolus, and Lupinus the fungus was ordinarily found first upon the base of the stem or on the primary root (Pl. 18, fig. C). On pansy (*Viola tricolor*) and phlox, where only very slight infections occurred, they were invariably found at the ends of the smallest fibrous roots.

The character of sporulation seemingly differs mostly in the variation of the appearance of the perithecial stage. Many workers have experienced failure or considerable difficulty in locating this stage and have therefore questioned the connection of the perithecia as described by Zopf with the chlamydospores of *T. basicola*. The association of the perithecia upon a large number of the different host plants observed in these tests with the chlamydospore stage of *T. basicola* is fairly convincing as to the correctness of Zopf's conclusions. The perithecia were found to be especially abundant upon *Cucumis maxima*, *Robinia pseudoacacia*, *Cytisus scoporius*, *Nicotiana tabacum*, and to a lesser extent upon a number of other hosts. Although it can not be so stated with certainty, the perithecial stage is apparently never produced in the same way on some host plants, as it is lacking in pure cultures of the fungus. The size, shape, number, and color of chlamydospores produced upon the various hosts differed to some extent. These differences appear to be determined in part by the location of these spore chains. When formed within the host cells, as is common in certain hosts, they were often restricted in their growth and were malformed. In other hosts such as *Cucumis* spp. and *Linaria* spp. they are commonly formed outside of the host cells and are large and uniformly shaped. The color of the chlamydospores varies from a deep blue-black, as on species of Cucumis, to a light brown, as on species of Nicotiana. The conidial spore form is only rarely seen on the living host, although it is produced early and profusely in culture media.

The addition of a large number of new host plants of *T. basicola*, while working with a comparatively small number of species, is taken to mean that the range of host species may perhaps be again doubled in time. Although its pathogenicity was questioned by earlier workers, it is shown that these conclusions were drawn from limited data and that these investigators used species which were either immune or relatively

resistant to attack. The vigorous pathogenicity of this organism on the more susceptible species and varieties can not be questioned. The vigor of the attack on certain susceptible varieties of tobacco at least is as striking as that of any known root disease of plants (Pl. 19). Since the fungus apparently spreads relatively slowly through the cell tissue, however, it seems apparent that soils must be abundantly infected and that a large number of local infections must occur before plants become badly diseased. Considering the wide range of hosts possessed by this fungus, it is possible that large economic losses, which are as yet relatively unknown, may be due to this fungus. In the case of tobacco alone it is certain that an average annual loss amounting to several millions of dollars occurs. Large losses also occur in the culture of violets (*Viola* spp.) and ginseng (*Panax quinquefolium*). Little or nothing is known about the extent of the damage which may be done to the various cultivated leguminous crops under field conditions. It is believed that the fungus will become a serious disease of the peanut (*Arachis hypogaea*) and cotton (*Gossypium* spp.), if it has not already become one.

SUMMARY

(1) *Thielavia basicola* Zopf is a fungus parasite attacking primarily members of the Leguminosae, Solanaceae, and Cucurbitaceae. Other families containing hosts of this fungus are Araliaceae, Bignoniaceae, Compositae, Convulvulaceae, Cruciferae, Hydrophyllaceae, Malvaceae, Orchidaceae, Oxalidaceae, Papaveraceae, Polemoniaceae, Portulaceae, Primulaceae, Scrophulariaceae, and Violaceae.

(2) Infection could not be obtained upon the following species of plants reported by others as hosts of *T. basicola: Phaseolus multiflorus, Nicotiana rustica, Scorzonera hispanica, Daucus carota, Apium graveolens, Beta vulgaris*, and *Pastinica sativa*.

(3) Thirty-nine species of plants have been reported by earlier investigators as hosts of *T. basicola*. Thirty-two of the thirty-nine reported hosts plants have been grown in soil infected with *T. basicola*, and infection obtained upon twenty-five of these plants. Of the seven upon which negative results in infection were secured, it is believed that all should be excluded from the list of hosts until further corroboratory evidence of infection is obtained. The remaining seven species could not be tested, owing to the difficulty of getting seeds or plants.

(4) Sixty-six new species of plants are added as hosts of *T. basicola*, of which twenty-eight are legumes, twenty are solanaceous plants, seven are cucurbits, and eleven belong to miscellaneous families.

(5) A great difference in the susceptibility of the various species exists; and where earlier workers have been inclined to doubt the parasitism of *T. basicola*, it appears to have been due to the fact that infection experiments were carried on with what are now known to be immune or very resistant plants.

(6) Some differences in point of attack and character of sporulation of *T. basicola* on different hosts have been noted. The common occurrence of the perithecial stage in close association with the chlamydospores of a number on different hosts is taken as good indirect corroboratory evidence of Zopf's connection of this form with *T. basicola*.

(7) The infection of nearly 100 different species of plants with *T. basicola* from tobacco is further evidence that no specialized races of this fungus appear to exist.

LITERATURE CITED

(1) ADERHOLD, Rudolf.
1905. Impfversuche mit Thielavia basicola Zopf. *In* Arb. K. Gsndhtsamt., Biol. Abt., Bd. 4, Heft 5, p. 463–465.

(2) BERKELEY, M. J., and BROOME, C. E.
1850. Notices of British fungi. *In* Ann. and Mag. Nat. Hist., s. 2, v. 5, p. 455–466, pl. 11–12.

(3) BURKHOLDER, W. H.
1916. Some root diseases of the bean. (Abstract.) *In* Phytopathology, v. 6, no. 1, p. 104.

(4) CHITTENDEN, F. J.
[1911.] Report [on investigations of sweet pea diseases]. *In* Sweet Pea Ann. 1911, p. 35–39.

(5) ———
1912. On some plant diseases new to, or little known in Britain. *In* Jour. Roy. Hort. Soc. [London], v. 37, pt. 3, p. 541–550.

(6) CLINTON, G. P.
1907. Root rot of tobacco, Thielavia basicola, (B. & Br.) Zopf. *In* Conn. Agr. Exp. Sta. 30th Ann. Rpt. [1905] /06, p. 342–368, fig. 14, pl. 29–32.

(7) GILBERT, W. W.
1909. The root-rot of tobacco caused by Thielavia basicola. U. S. Dept. Agr. Bur. Plant Indus. Bul. 158, 55 p., 5 pl. Bibliography, p. 44–48.

(8) KILLEBREW, J. B.
1884. Report on the Culture and Curing of Tobacco in the United States. 286 p., illus. Washington, D. C. Published by U. S. Dept. Int. Census Office.

(9) KIRCHNER, Oskar.
1906. Die Krankheiten und Beschädigungen unserer landwirtschaftlichen Kulturpflanzen ... Aufl. 2, 675 p. Stuttgart.

(10) MASSEE, G. E.
1884. Description and life-history of a new fungus, Milowia nivea. *In* Jour. Roy. Micros. Soc. [London], s. 2, v. 4, p. 841–845.

(11) ———
1912. A disease of sweet peas, asters, and other plants. *In* Roy. Gard. Kew, Bul. Misc. Inform., 1912, no. 1, p. 44–52.

(12) O'GARA, P. J.
1915. Occurrence of Thielavia basicola as a root parasite of watermelons in the Salt Lake Valley of Utah. *In* Science, n. s. v. 42, no. 1079, p. 314.

(13) PEGLION, Vittorio.
1897. Marciume radicale delle piantine di tabacco causato dalla Thielavia basicola, Zopf. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 3, p. 580–584.

(14) ROSENBAUM, Joseph.
1912. Infection experiments with Thielavia basicola on ginseng. *In* Phytopathology, v. 2, no. 5, p. 191–196, pl. 18–19.

(15) SELBY, A. D.
1896. Investigations of plant diseases in forcing house and garden. Ohio Agr. Exp. Sta. Bul. 73, p. 221–246, illus.
(16) ——
1904. Tobacco diseases and tobacco breeding. Ohio Agr. Exp. Sta. Bul. 156, p. 87–114, 3 fig., 8 pl.
(17) ——
1910. A brief handbook of the diseases of cultivated plants in Ohio. Ohio Agr. Exp. Sta. Bul. 214, p. 307–456. Literature referred to, 7 p. at end of bulletin.
(18) SMITH, Erwin F.
1899. Wilt disease of cotton, watermelon, and cowpea (Neocosmospora nov. gen.) U. S. Dept. Agr. Div. Veg. Physiol. and Path. Bul. 17, 72 p., 10 pl. (1 col.).
(19) SORAUER, Paul.
1895. Über die Wurzelbräune der Cyclamen. *In* Ztschr. Pflanzenkrank., Bd. 5, Heft 1, p. 18–20.
(20) SOROKIN, Nicolas.
1876. Ueber Helminthosporium fragile sp. n. *In* Hedwigia, Bd. 15, No. 8, p. 113.
(21) TAUBENHAUS, J. J.
1914. The diseases of the sweet pea. Del. Agr. Exp. Sta. Bul. 106, 93 p., tab., 43 fig. References, p. 88–93.
(22) THAXTER, Roland.
1892. Fungus in violet roots. *In* Conn. Agr. Exp. Sta. Ann. Rpt. 1891, p. 166–167.
(23) ZOPF, Wilhelm.
1876. [Ueber Thielavia basicola, einen endophytischen Parasiten in den Wurzeln des Senecio elegans.] *In* Verhandl. Bot. Ver. Brandb., Jahrg. 18, p. 101–105.
(24) ——
1891. Über die Wurzelbräune der Lupinen, eine neue Pilzkrankheit. *In* Ztschr. Pflanzenkrank., Bd. 1, p. 72–76, illus.

PLATE 18

Fairly typical diseased spots and lesions caused by *Thielavia basicola* on various host plants. Some of the infected areas are indicated by arrows and braces:

Fig. A.—*Citrullus vulgaris* (citron).

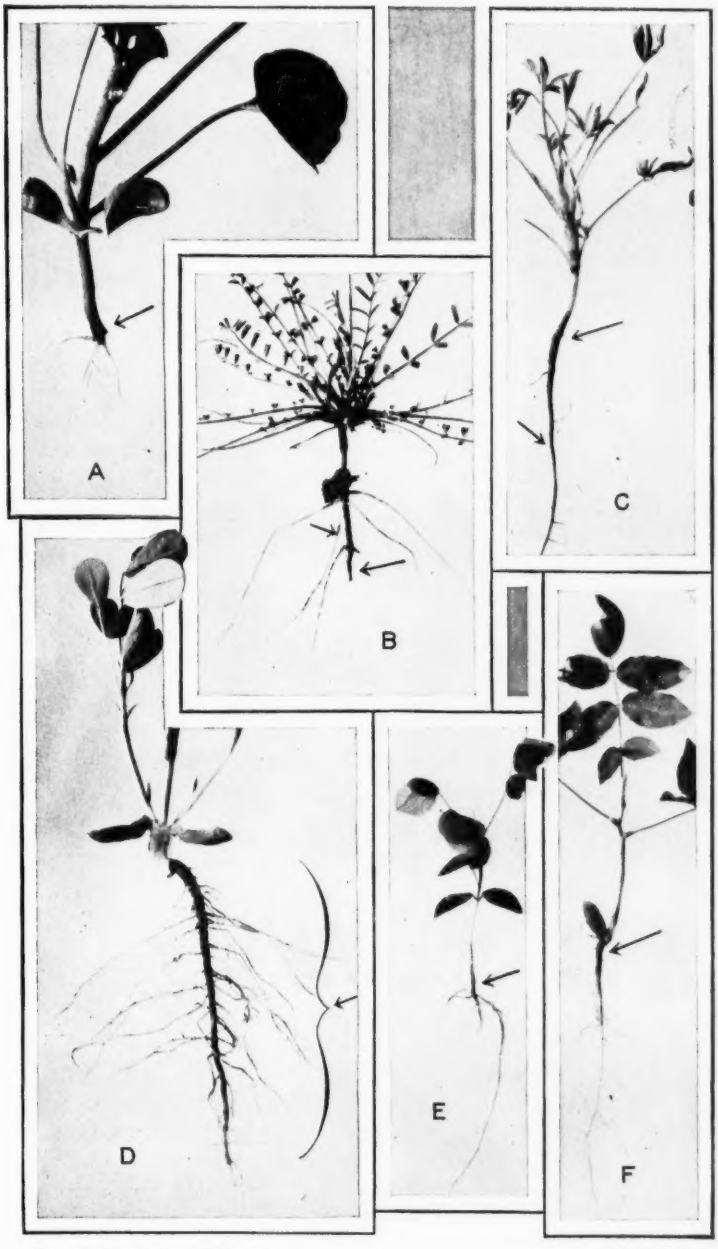
Fig. B.—*Onobrychis viciaefolia* (sainfoin).

Fig. C.—*Lupinus luteus* (yellow lupine).

Fig. D.—*Arachis hypogaea* (peanut).

Fig. E.—*Robinia pseudoacacia* (black locust).

Fig. F.—*Sclotis chinensis* (wistaria).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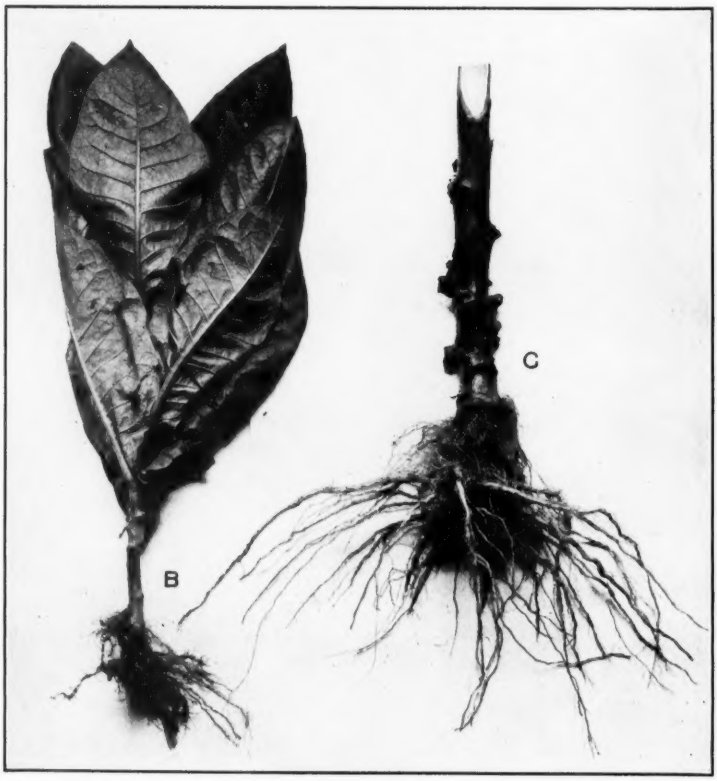
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C

PLATE 19

Fig. A.—Part of a field infected with *Thielavia basicola* in foreground, with newer soil planted to tobacco in the background, illustrating the marked pathogenic powers of this organism.

Fig. B.—A tobacco plant showing diseased roots from infected soil.

Fig. C.—Healthy roots from uninfected soil of a semiresistant type of tobacco (Connecticut Havana). Figures B and C show the relative growth of plants and amount of root system after equal care in removing roots from the soil.